Genetics 371B, Autumn 1999

Introductory Genetics

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The mechanism of inheritance...

Some early hypotheses:

- **Predetermination** e.g., the homunculus theory

- **Blending** of traits

Introducing a more systematic approach…
Gregor Mendel (1822–1884) and his experiments with garden pea

But first:

Choosing a **model organism**

- What is it?

- Why bother?

- Features of a good model organism:

- Some commonly used model organisms:

Mendel's organism of choice: **garden pea**

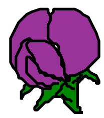
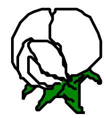
His question: If a pair of plant lines showing a clear character difference are crossed, will the progeny show an intermediate **phenotype**?

He established **true-breeding** lines…

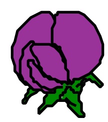
…that showed character differences

Made crosses (matings) between each pair of lines

Example:
Character:
Phenotypes:

 x 

↓

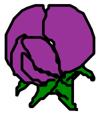
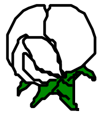
"F_1" 

F1 x F1

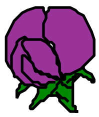
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"F_2"

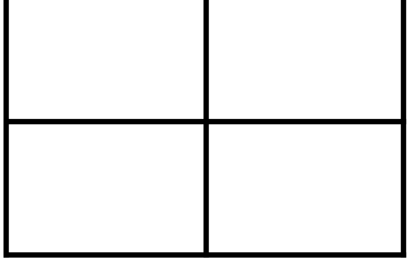
Interpreting Mendel's experiment

Parents:  

Gametes:

F1 progeny: 

Gametes:



F2 progeny:

Conclusions:

1. Determinants are **particulate**
2. They occur in **pairs**; one member may be **dominant**
3. Determinants **segregate randomly** into gametes

Prediction: The F2 "Purple" class consists of two subclasses:

Testing the prediction:

What Mendel did:

What we would do today (hindsight!):

Generality of Mendel's first law:
(Not just for pea plants!)

- Fruit fly (*Drosophila melanogaster*)

  Normal (brown) body x black body

- Mice

  Agouti x Black

- Humans

  Albinism

Pedigree analysis

- What are pedigrees?

- Why bother with them?

- Constructing pedigrees

  "The **inability** to smell methanethiol is a **recessive** trait in humans. Ashley, Perry, and Gus are three smelling children of Erin (a non-smeller) and Darren (a smeller). Perry's only child is a non-smeller boy. Construct a pedigree for this family, indicating the genotypes where possible."

To be continued…

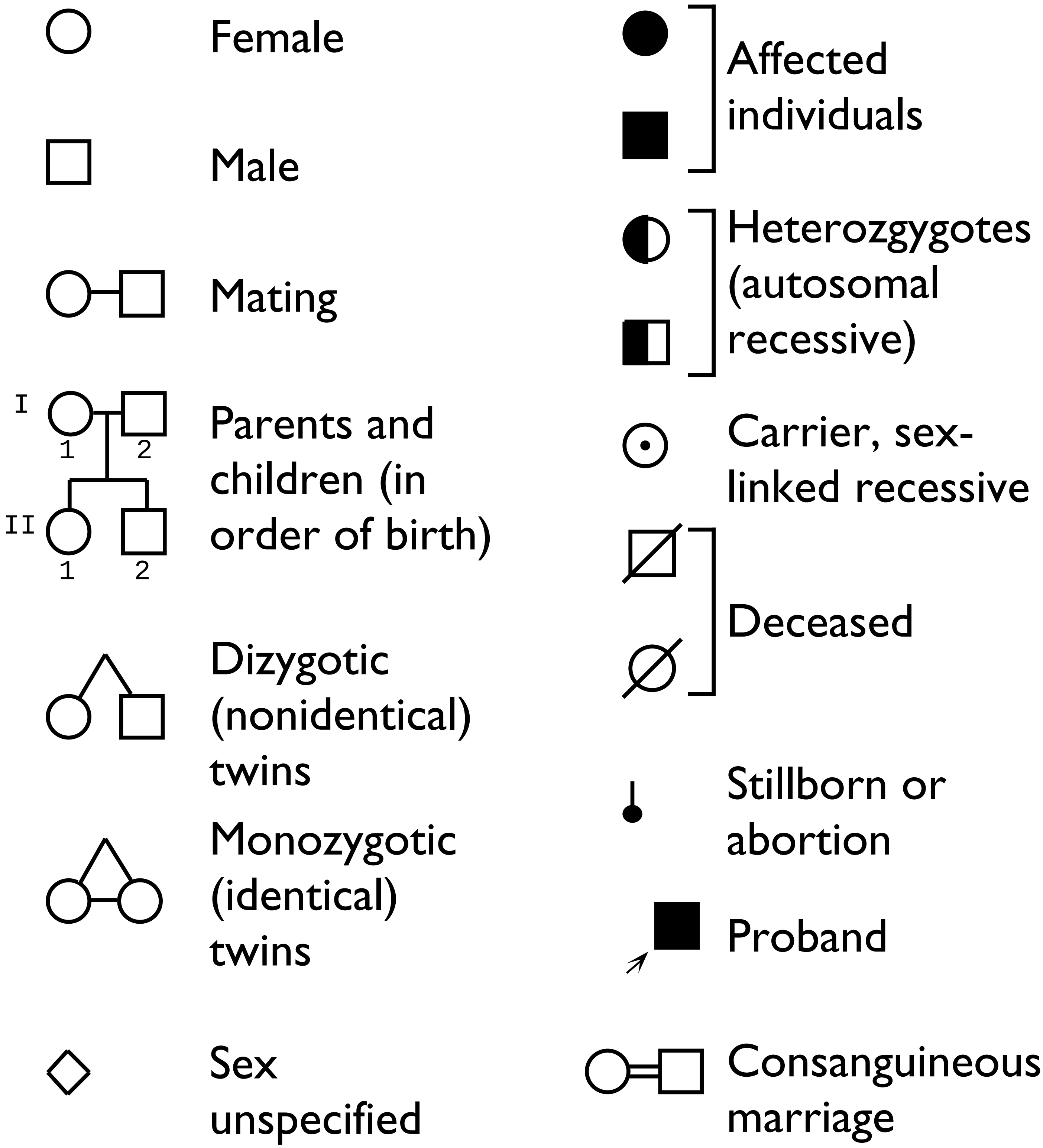
Complications

- Expressivity

- Penetrance

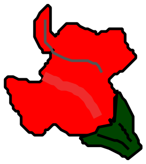
Do all human traits show simple Mendelian inheritance?

Commonly used pedigree symbols



Modified monohybrid ratios

- Snapdragon flower color



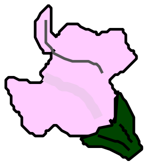
Red flowers

x



White flowers

↓

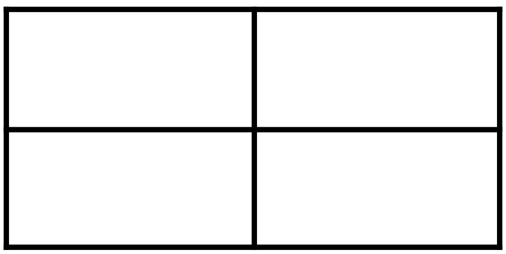


Pink flowers

Blending of determinants??

How to test?

Prediction?



. . . a case of **incomplete dominance**

Incompletely dominant or **recessive?**

. . . in the eye of the beholder?

e.g., Tay Sachs disease

Symptoms:

- extreme sensitivity to noise
- muscle weakness
- cherry-red spot on retina

Affected individuals rarely survive past childhood

Defect –

Overt phenotype . . .

At the biochemical level . . .

Co-dominance

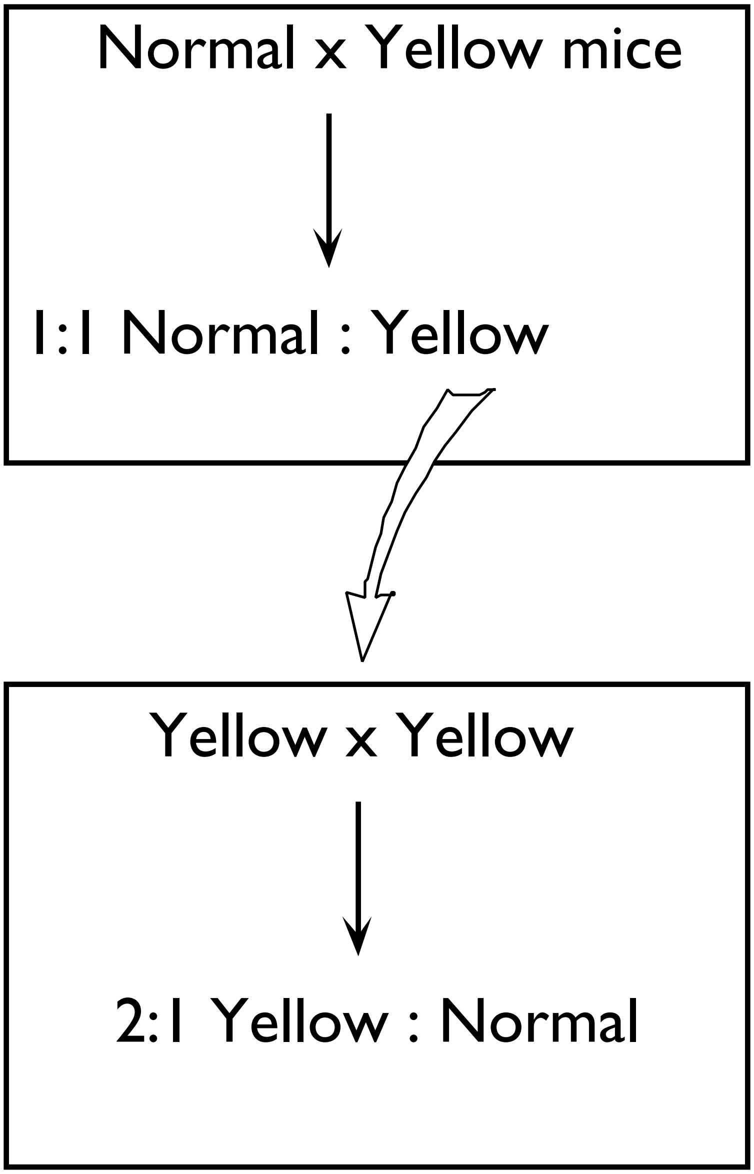
e.g., ABO blood group

Three possible alleles: A, B, or O

Looking at 3 different “crosses”:

AA x BB	AA x OO	BB x OO	⇦ Parental genotype
↓	↓	↓	
AB	A	B	⇦ Progeny phenotype
			⇦ Progeny genotype?

The curious case of the yellow mice



Interpreting:

- Which allele is dominant?
- Parental genotypes?

What's missing in F2?

The physical basis of Mendelian genetics

- 1902: Boveri and Sutton, “Chromosome theory of inheritance”

- Chromosomes

- Diploid vs. haploid chromosome number

What’s in a chromosome?

- Protein

- DNA (deoxyribonucleic acid)

  Subunit: Ribose + Phosphate + base

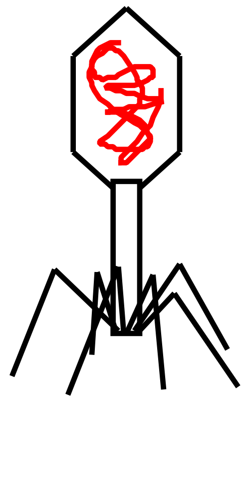
  Base: Adenine, Cytosine, Guanine, Thymine

DNA as the molecule of inheritance

The Hershey-Chase experiment

- **Question:** What is passed on from one generation to the next, **protein** or **DNA**?

- **Model organism:** Bacteriophage T2



- **The experiment**

Bacteriophage with radioactive **DNA**

Bacteriophage with radioactive **protein**

Infect bacteria (E. coli)

Do progeny virus have radioactive **DNA?**

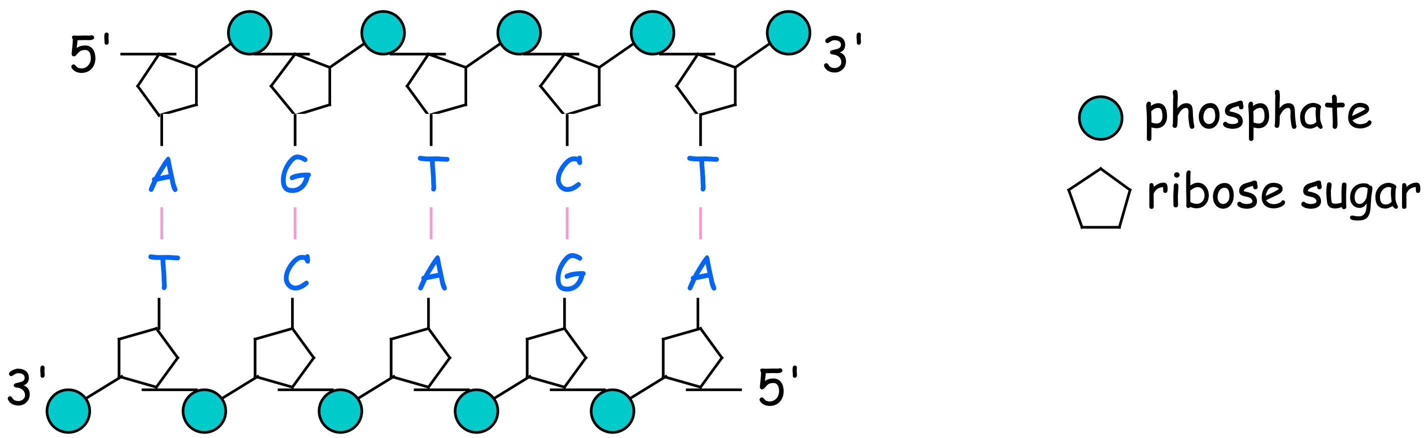
Do progeny virus have radioactive **protein?**

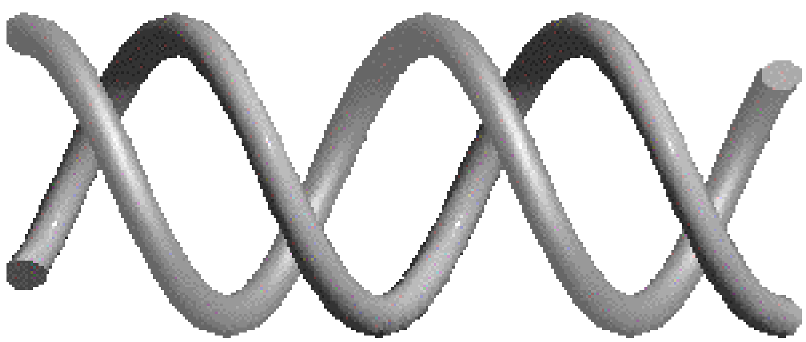
- **Conclusion:**

The structure of DNA

- Backbone
- Pairing







What holds the helices together?

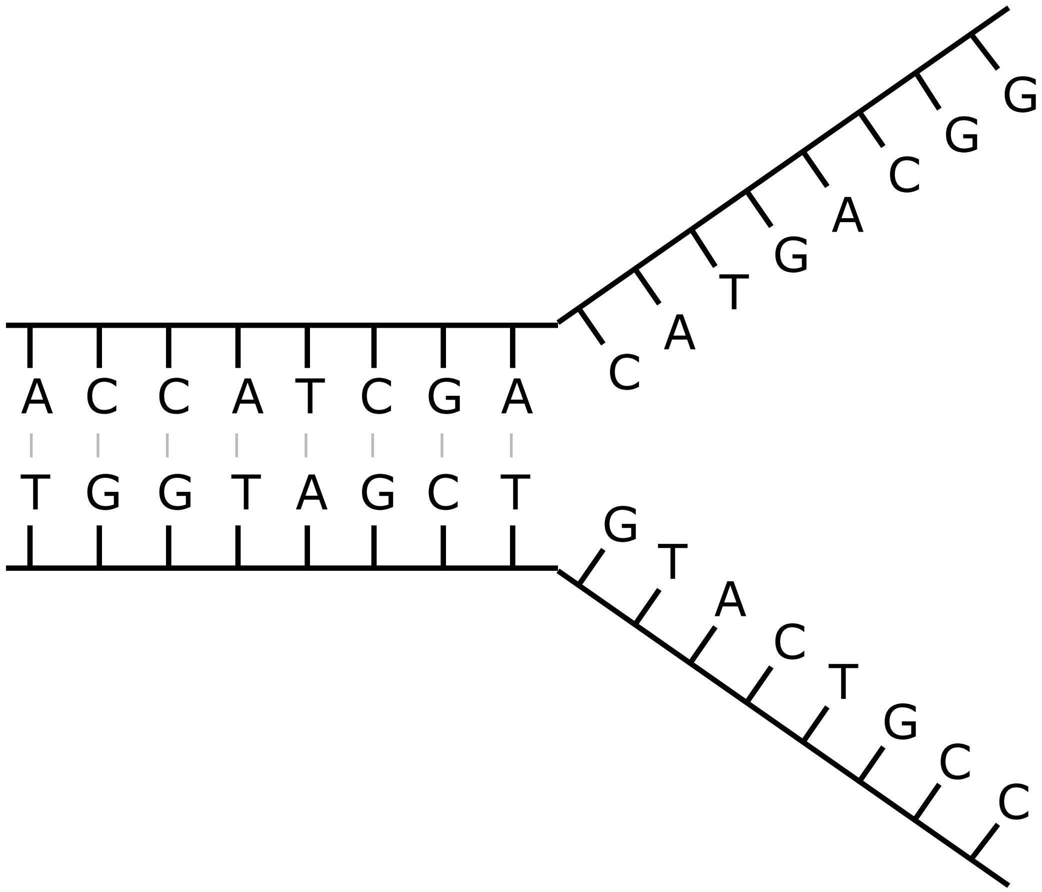
Length measure (double-stranded DNA):

Human genome:

What are alleles?

The cell cycle

DNA replication



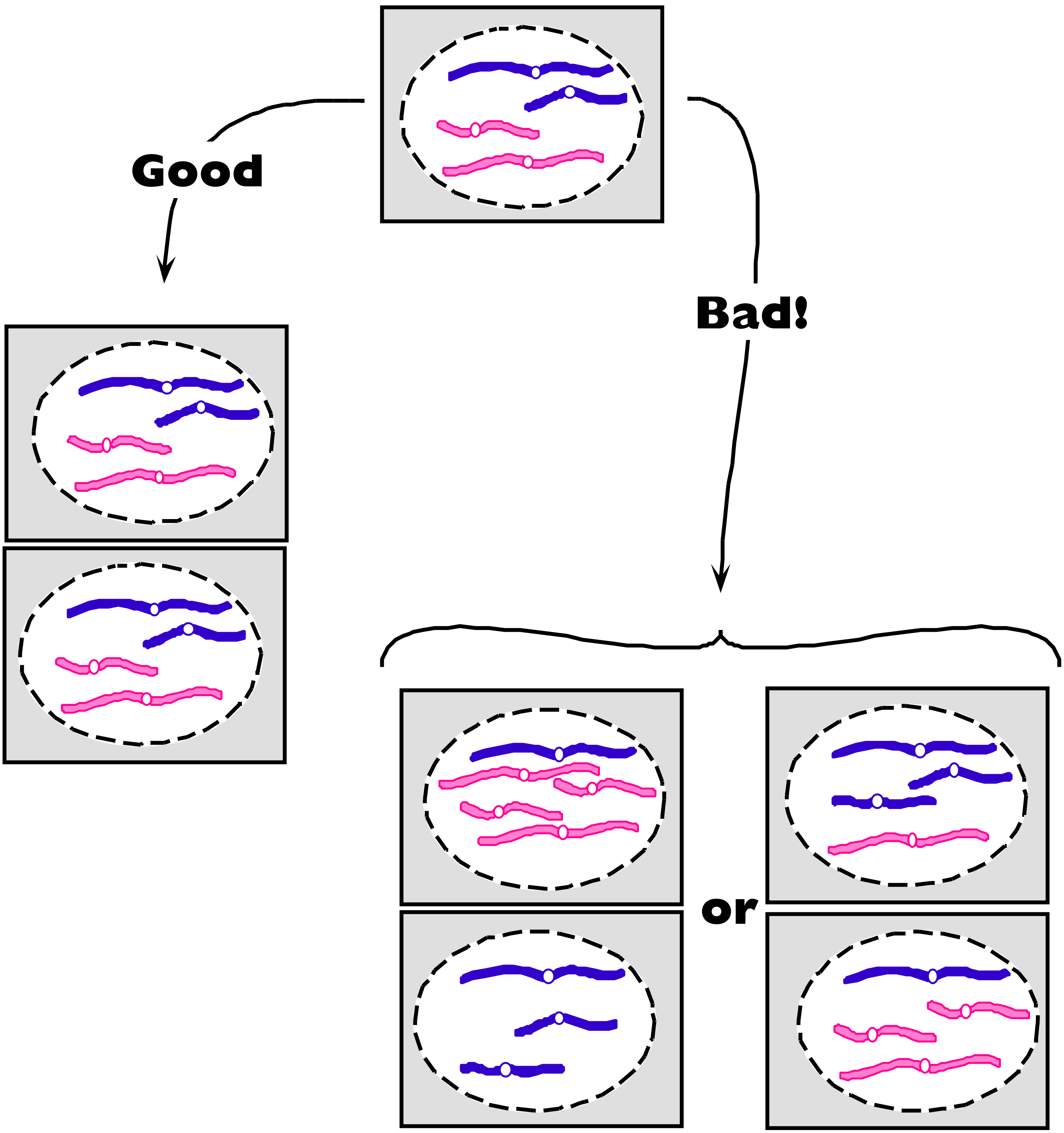
Cell division:

What happens to the chromosomes depends on the goal of the division

- to make more "vegetative" cells:

- to make gametes:

Mitosis – Partitioning replicated chromosomes

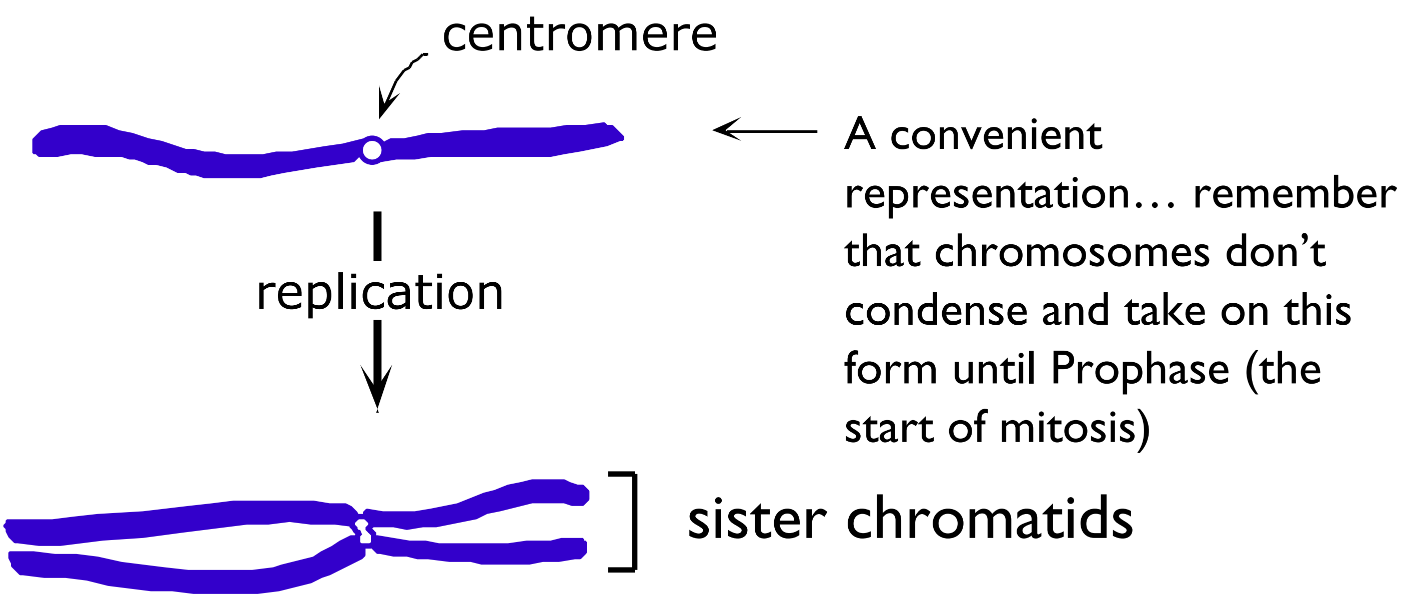


The problem: Partitioning replicated chromosomes so that each daughter cell gets one copy of each chromosome

The solution

After replication of a chromosome…

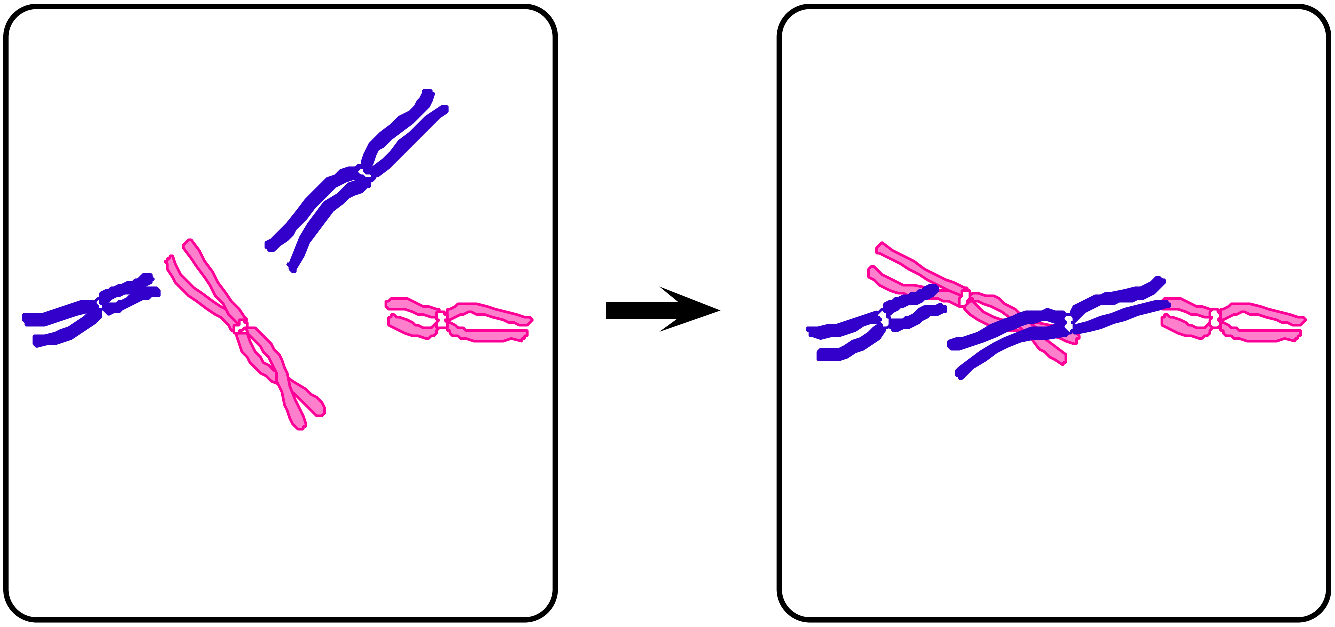
- hold the two **sister chromatids** together
- target them to opposite poles
- then separate the sisters



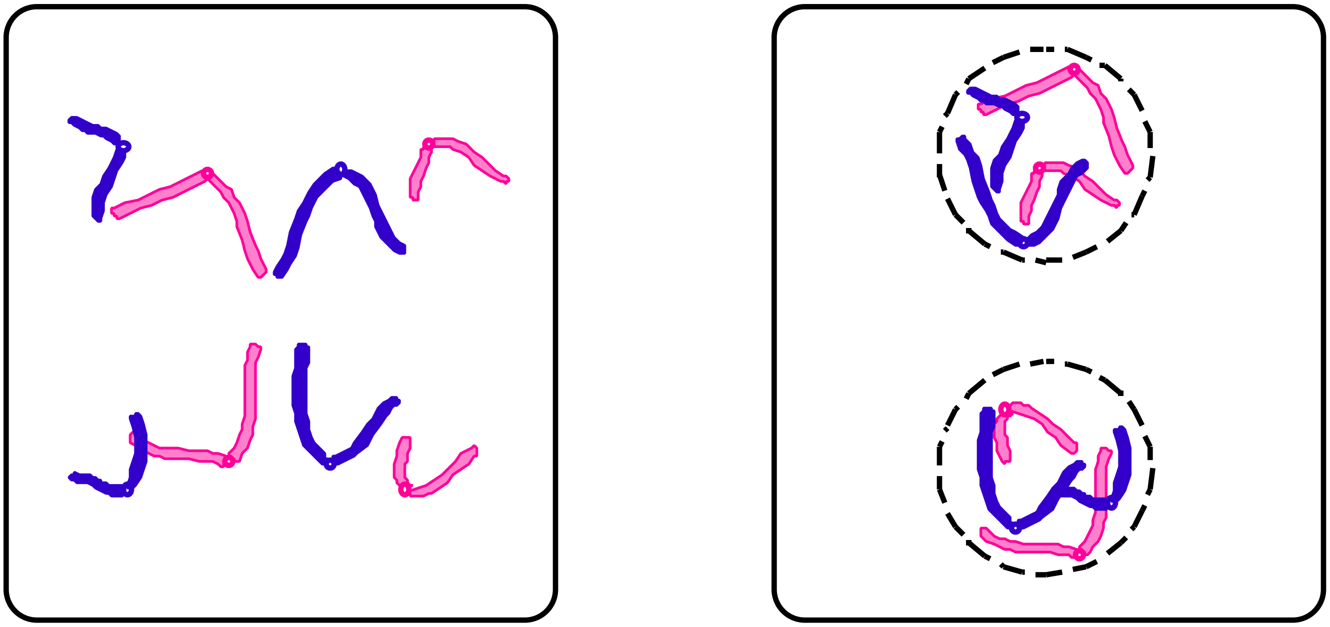
At Metaphase . . .

Chromosomes line up at cell's "equatorial plate"

Mechanism? Spindle fibers exerting tension on kinetochores



Once all the chromosomes are lined up…



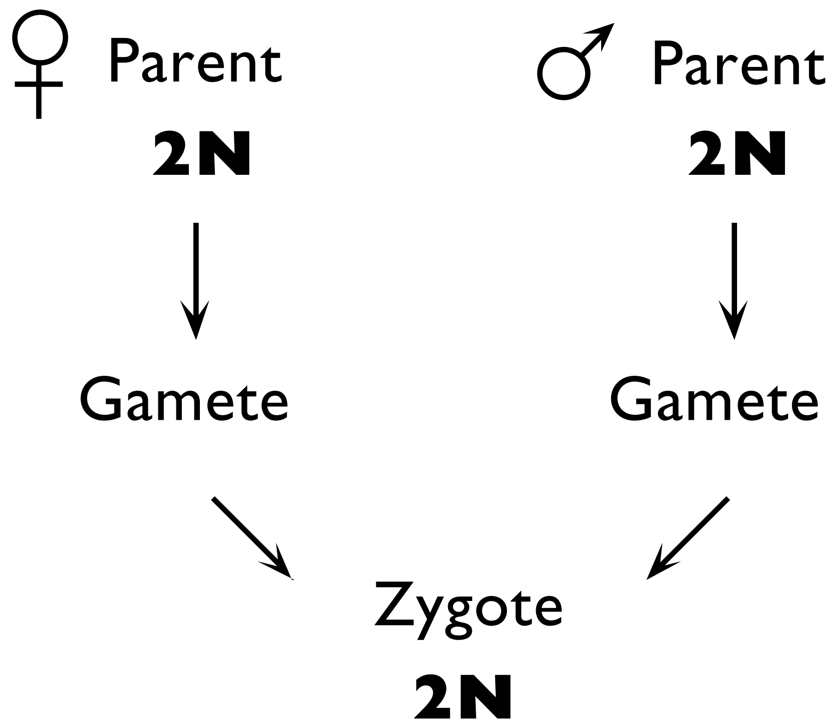
Anaphase **Telophase**

What kinds of defects would make mitosis go haywire?

Meiosis and the Chromosome theory

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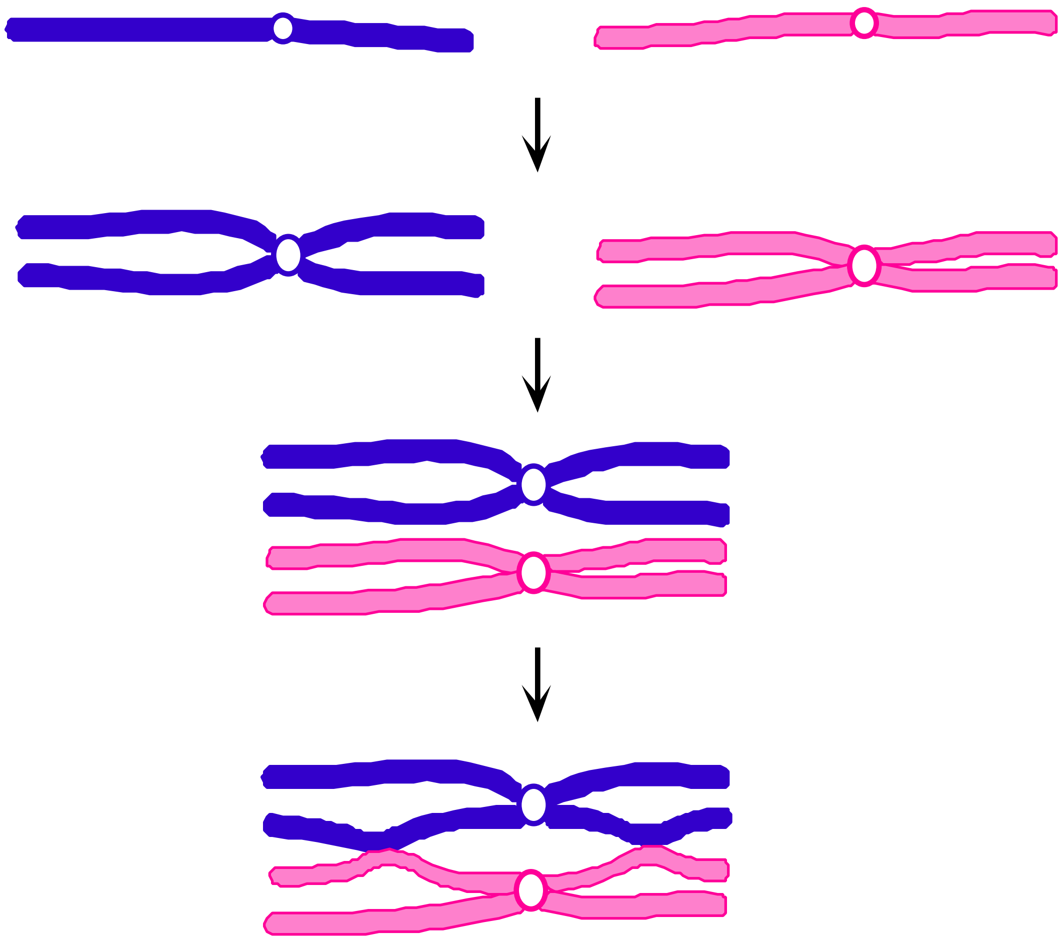
Meiosis - making **haploid** gametes from **diploid** cells



The problem: ensuring that homologs are partitioned to separate gametes

The solution

- hold homologous chromosomes together by **synapsis** and **crossing over**
- target homologs to opposite poles
- then separate the homologs



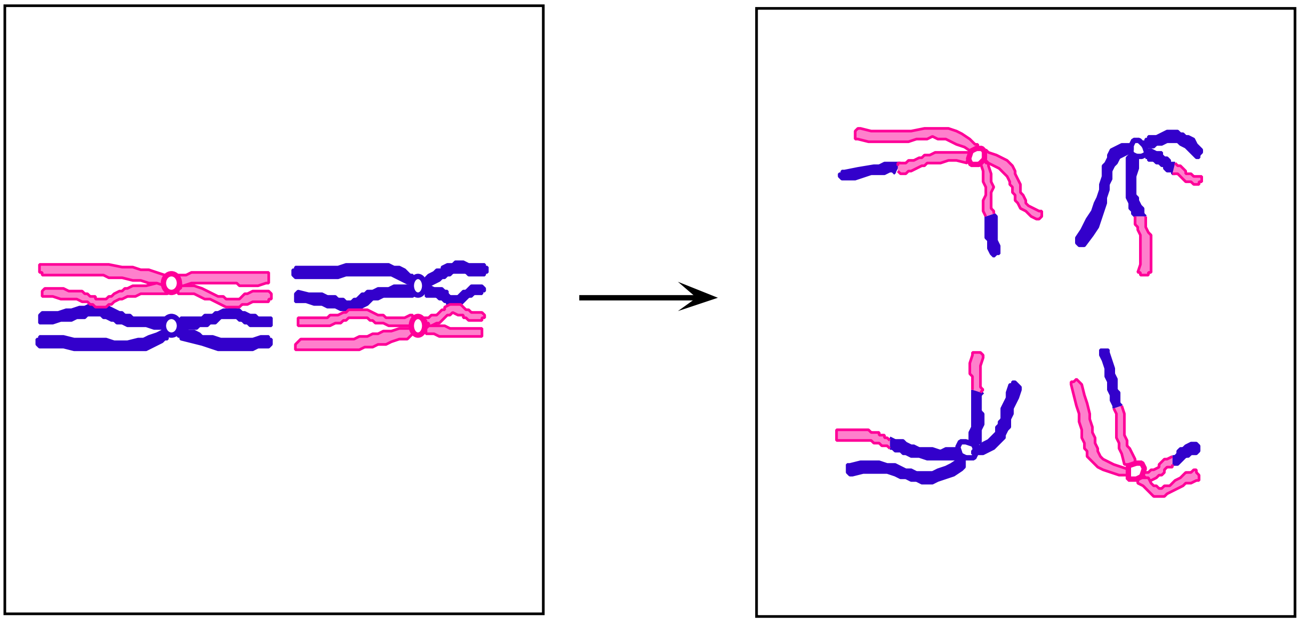
Meiosis proceeds in two steps:

- Meiosis I — **“reductional division**”

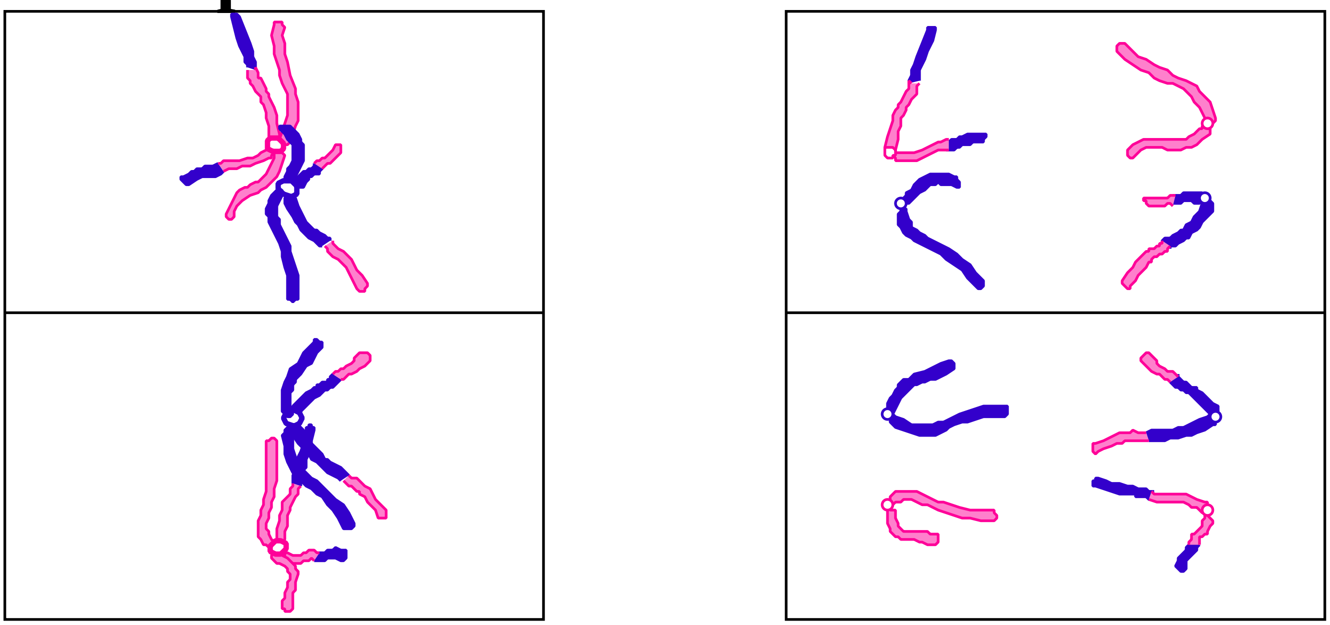
- Meiosis II — **“equational division”**

Metaphase I

Anaphase I



Metaphase II



The chromosome theory of inheritance

Based on the congruence of **determinant behavior** (Mendel) and **chromosome behavior** (cytology)

The essence of the theory:

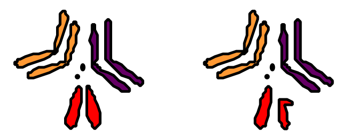
Proof- Based on tests of **predictions**:

- transmission of traits should parallel the segregation of specific chromosomes

- if chromosome segregation is altered the transmission of determinants should be altered also

Thomas Hunt Morgan, 1909: Test of the first prediction - in *Drosophila*

Chromosomes of *Drosophila melanogaster:*



Red eyes	white eyes	white eyes	Red
♀ × ♂		♀ × ♂	

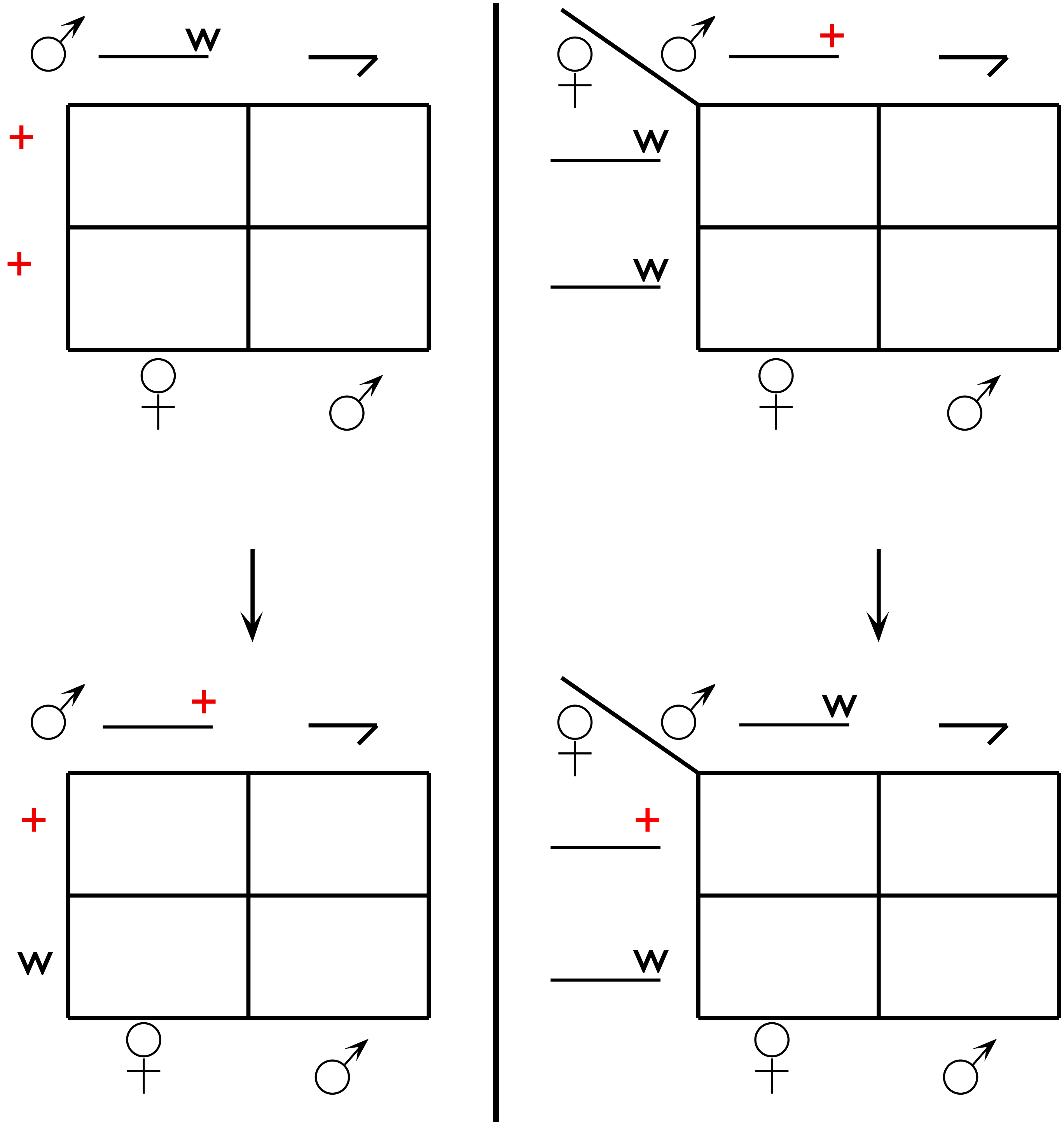
↓ ↓

F_1: ♀ ♂ | F_1: ♀ ♂

↓ ↓

F_2: ♀ ♂ | F_2:

Morgan's interpretation:



Conclusion:

Calvin Bridges' experiments with *exceptional progeny*: Test of the 2nd prediction

white eyes ♀ x Red ♂

↓

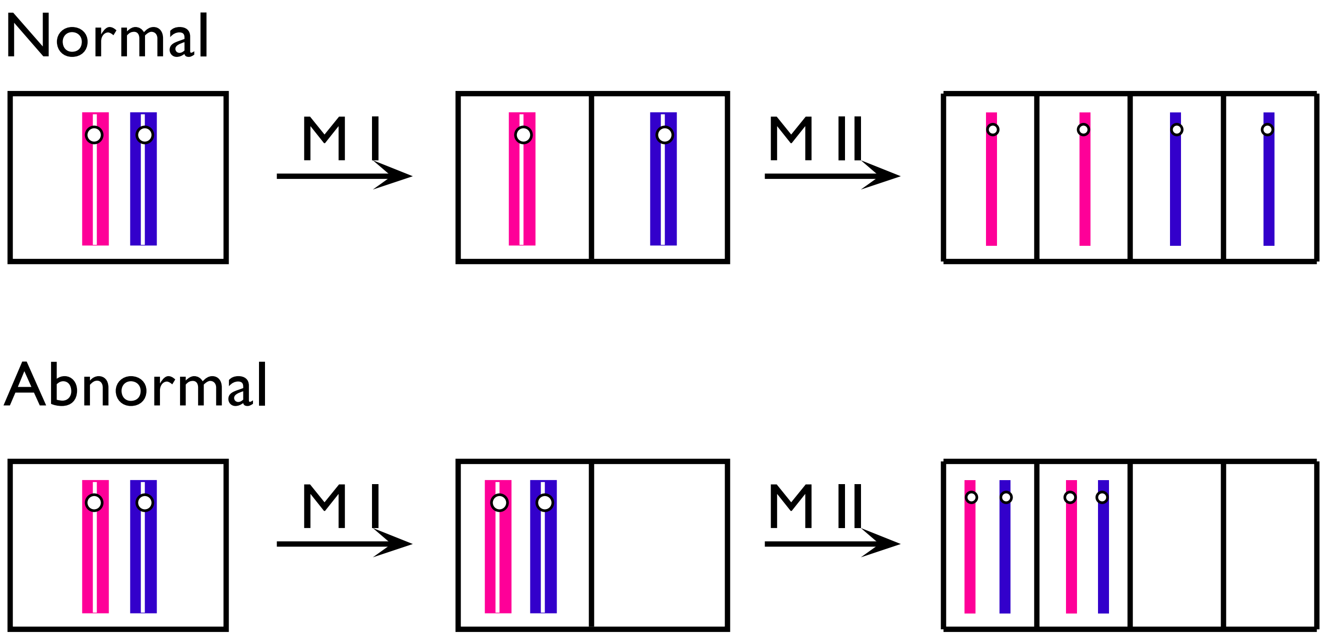
Expect:

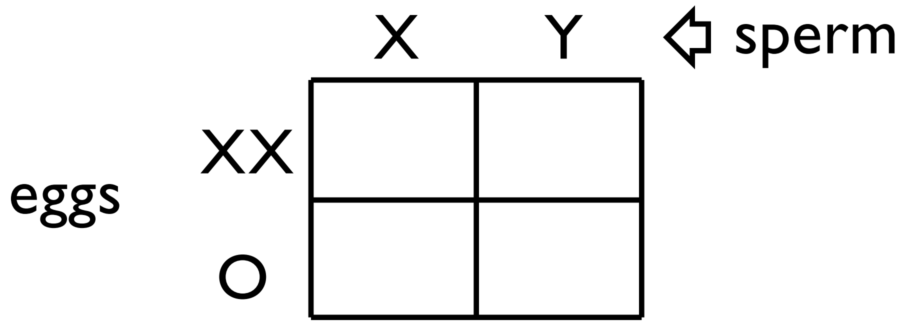
Occasionally got:

["primary exceptional progeny"]

Explanation?

Rare errors in meiosis ⇨ mis-segregation of chromosomes





Conclusions

1. Determinants are on chromosomes

2. In Drosophila, two X = female
(one X = male)

Sex determination

	♀ Female	♂ Male
Fruit fly		
Humans		
Birds		

Possibilities

Y ⇨ male

XX ⇨ female

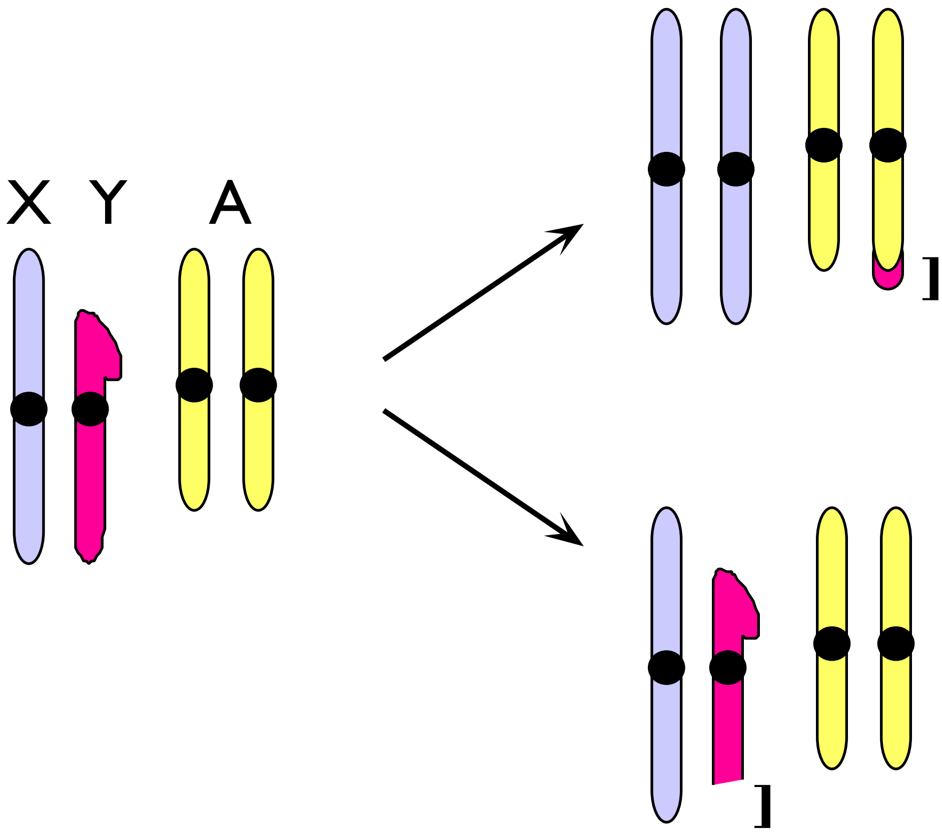
In humans, the **presence of a Y chromosome** makes a male:

Klinefelter syndrome: XXY

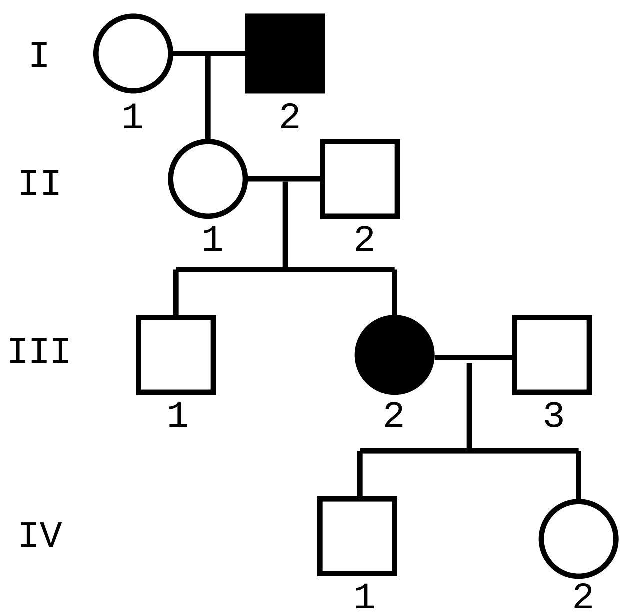
Turner syndrome: XO

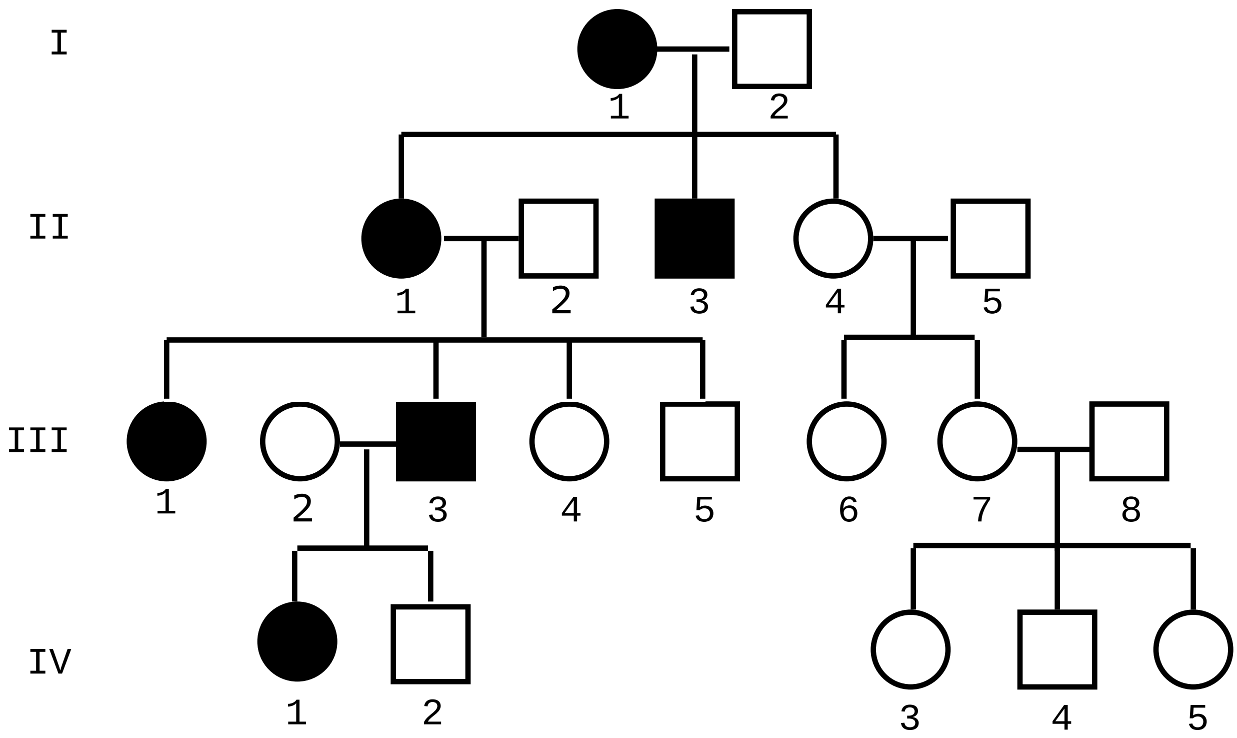
How does the Y chromosome cause male-ness?

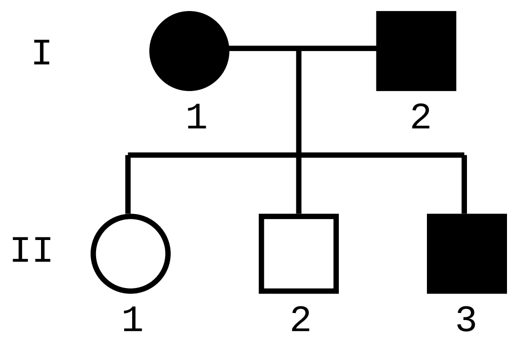
“TDF” (testis-determining factor) aka SRY gene on the Y chromosome…



- Analyzing pedigrees
  - The process
  - An assumption:
  - The result
- **Examples**
  For each of the following pedigrees, can you decide whether the trait is dominant or recessive?





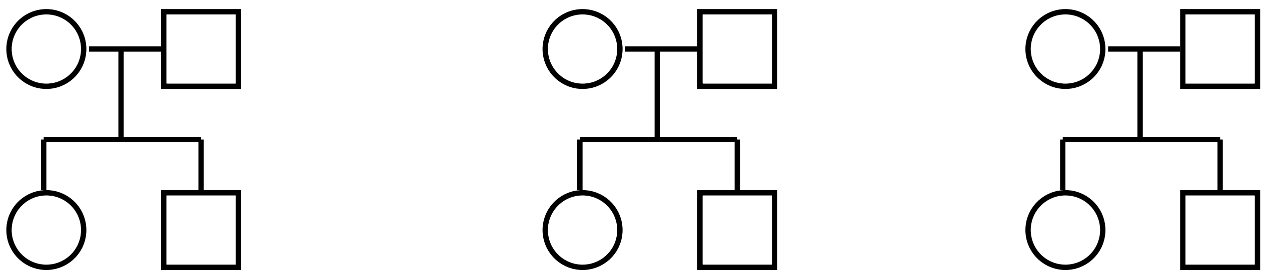


Is this a recessive trait?

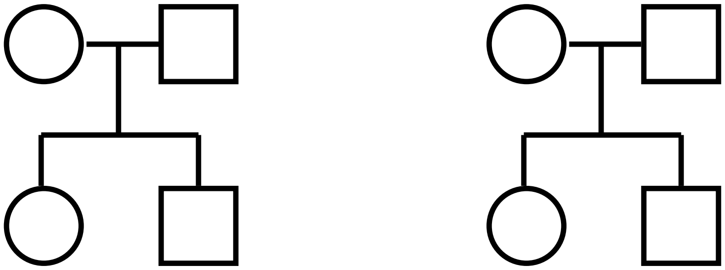
Sex-linked traits

- X-linked recessive

  Consider these pedigrees (to be filled in)



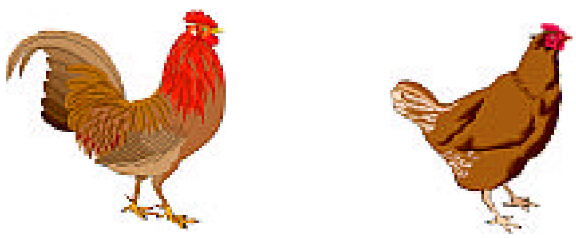
- X-linked dominant



- What would you predict for a Y-linked trait?

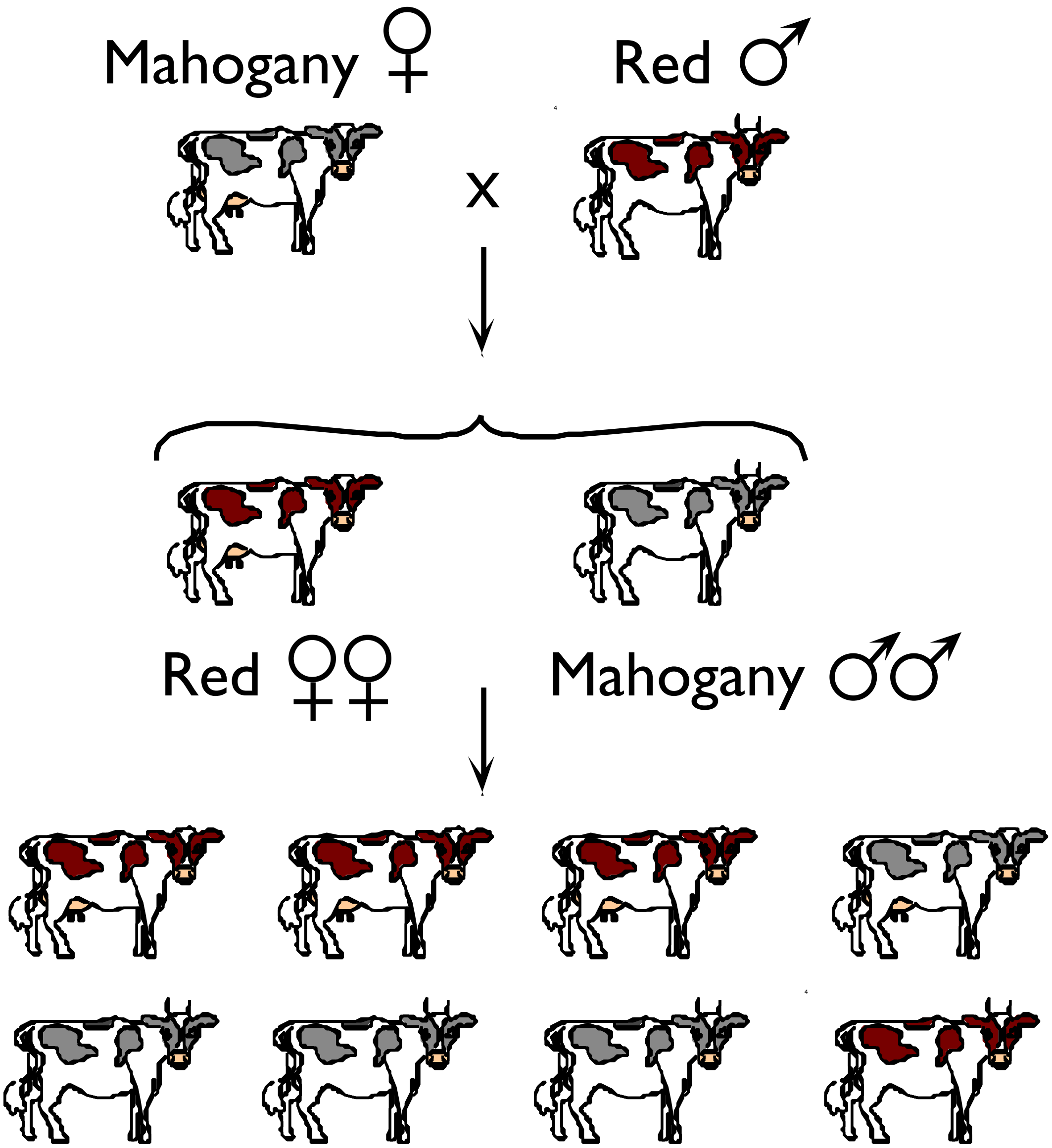
Sex-limited inheritance

e.g., hen-feathering in chicken



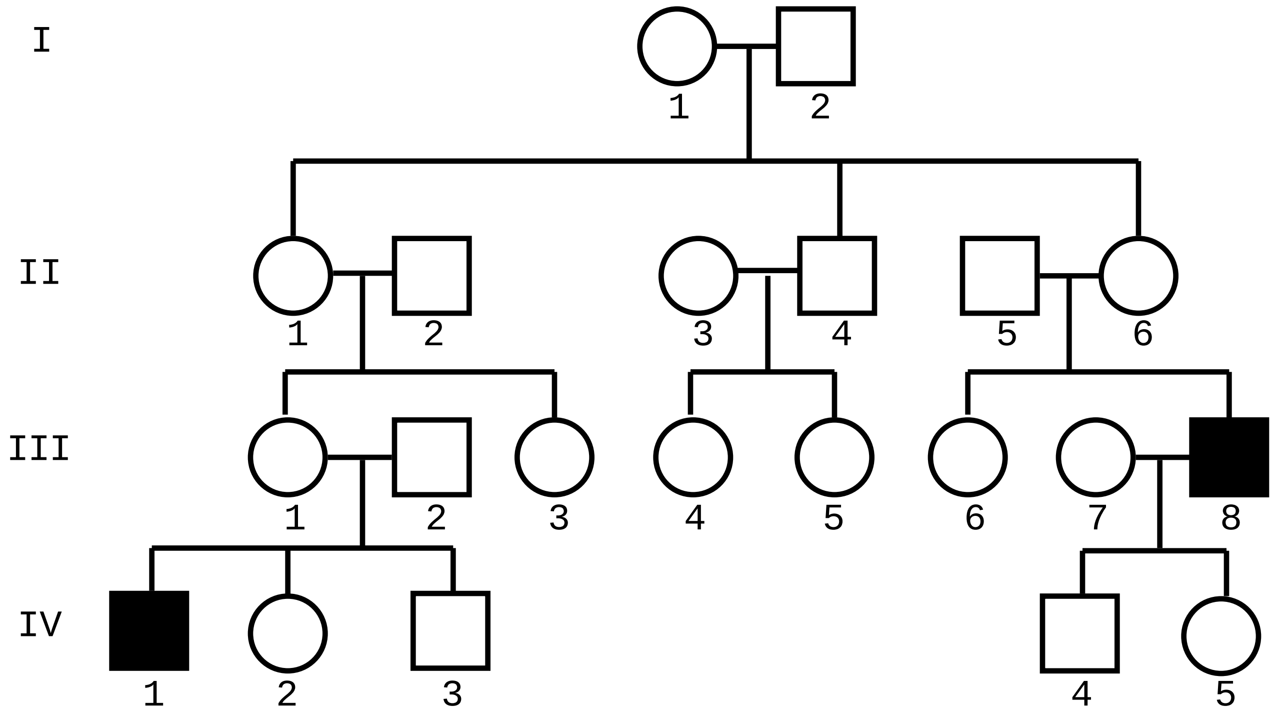
Genotype	Hen-feathered? ♀	♂
HH		
Hh		
hh		

Sex-influenced inheritance

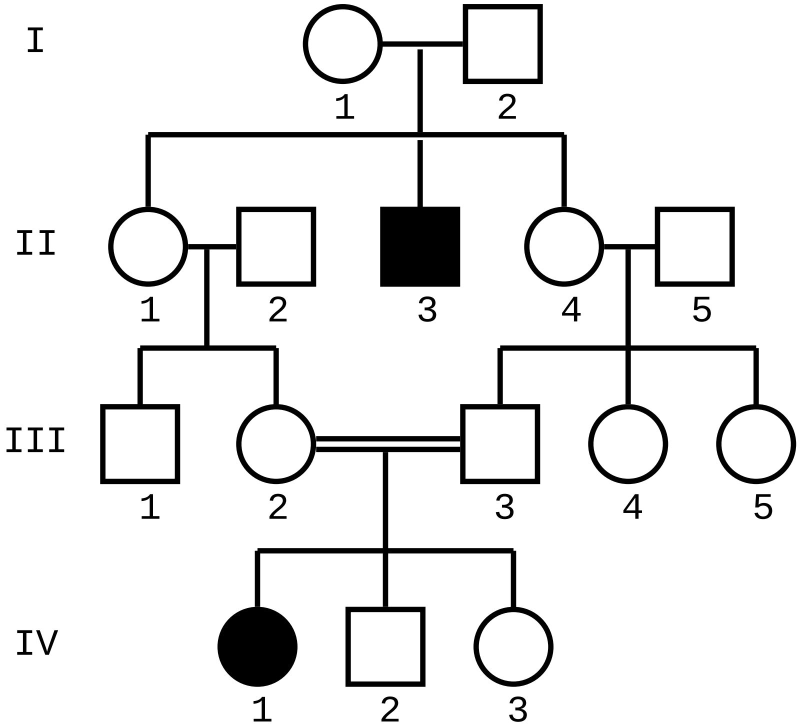


For each of the following pedigrees, which modes of inheritance can you eliminate, and why? (Assume complete expressivity and penetrance; also assume that the trait is rare and that unless indicated otherwise, there is no inbreeding.)

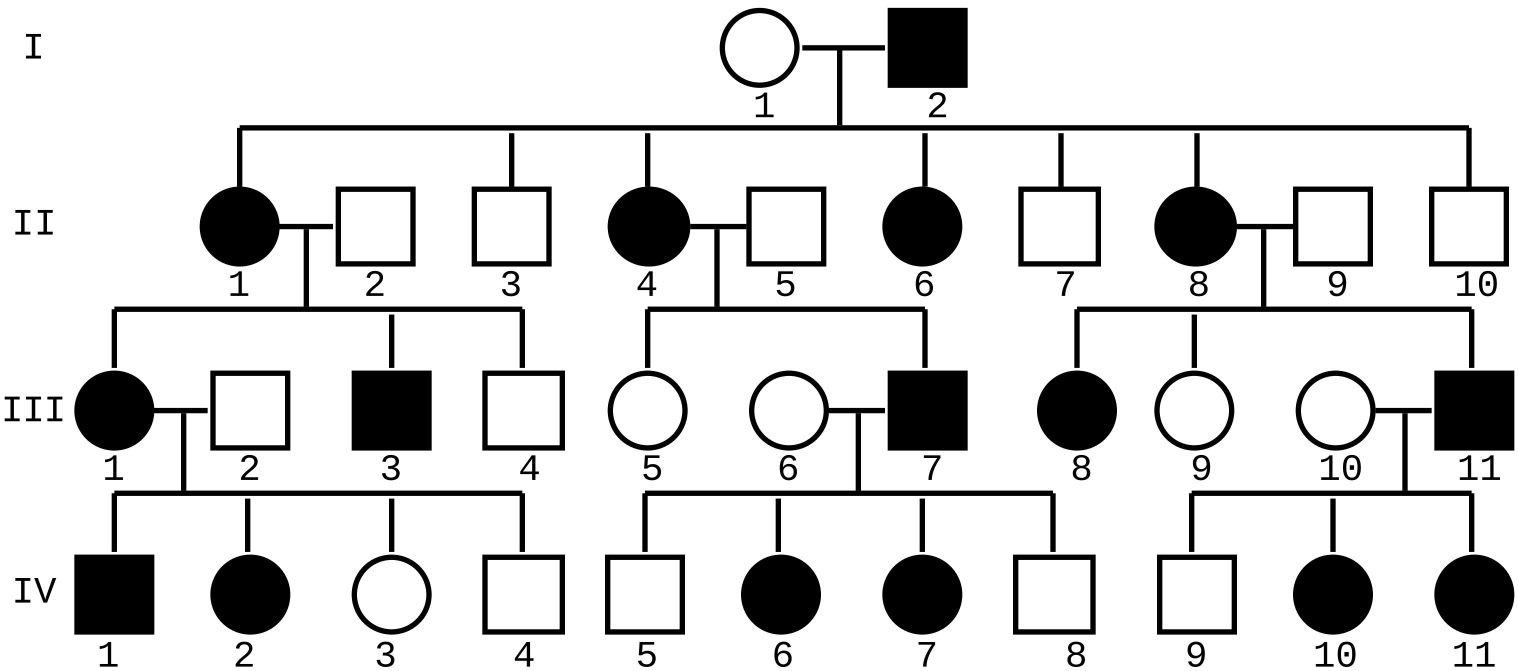
(A)



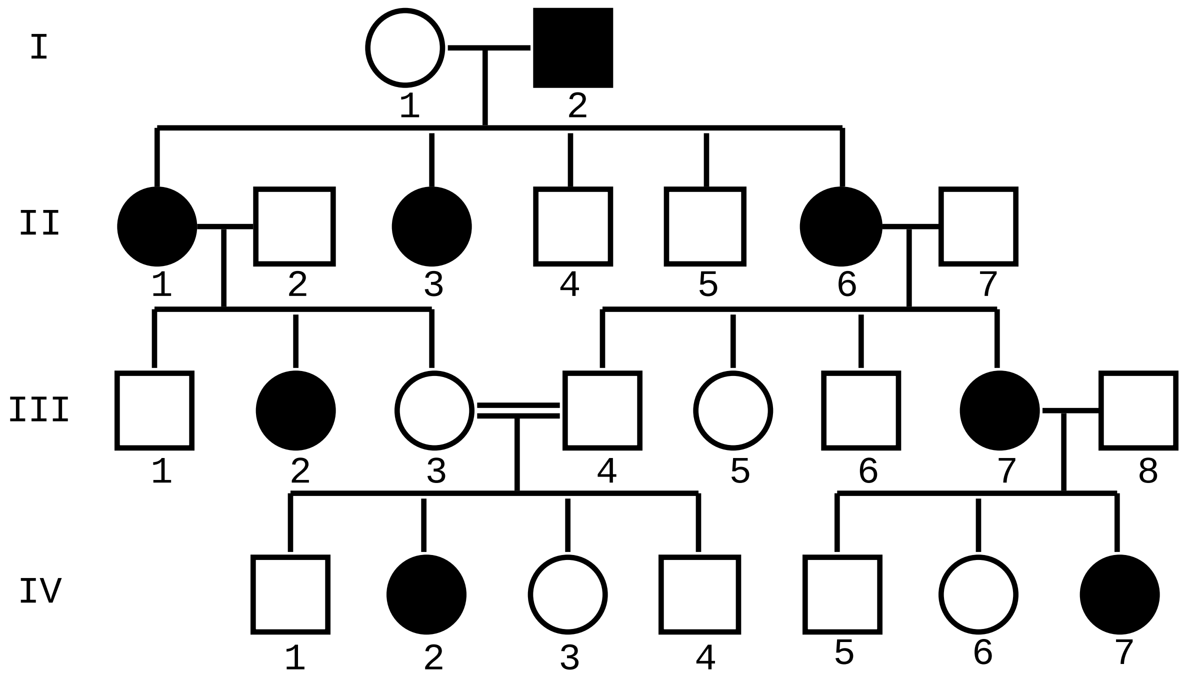
(B)


I
1 2
II
1 2 3 4 5
III
1 2 3 4 5
IV
1 2 3

(C)


I
1 2
II
1 2 3 4 5 6 7 8 9 10
III
1 2 3 4 5 6 7 8 9 10 11
IV
1 2 3 4 5 6 7 8 9 10 11

(D)



Independent assortment

Based on what we know about meiosis…expect **random segregation** of chromosomes

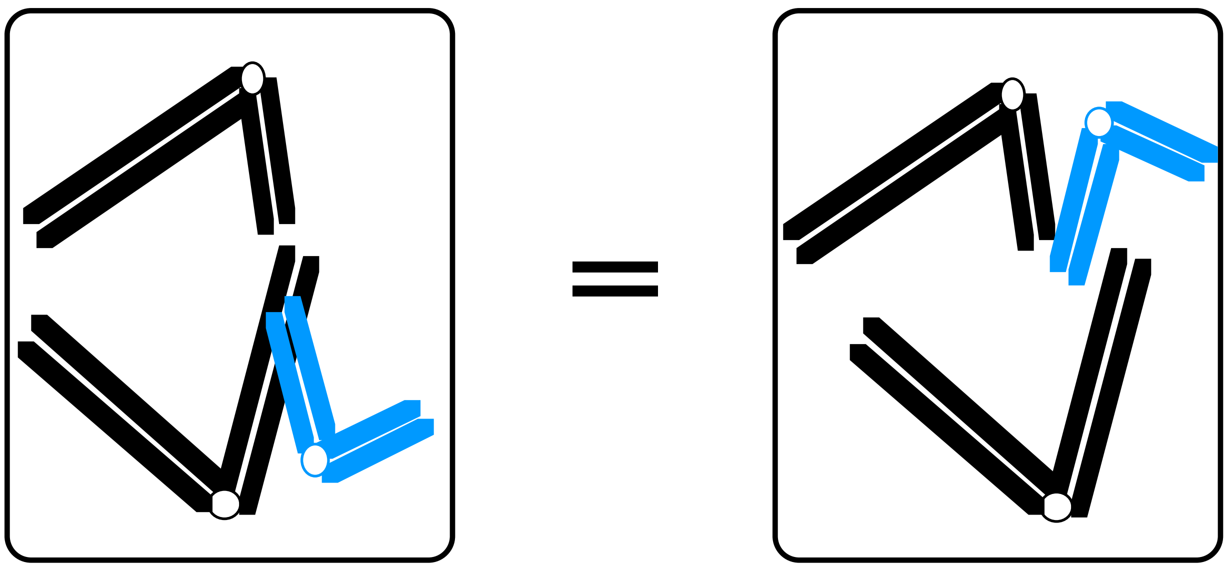
Evidence

Meiosis in grasshopper testes

- One heteromorphic chromosome pair; one unpaired chromosome

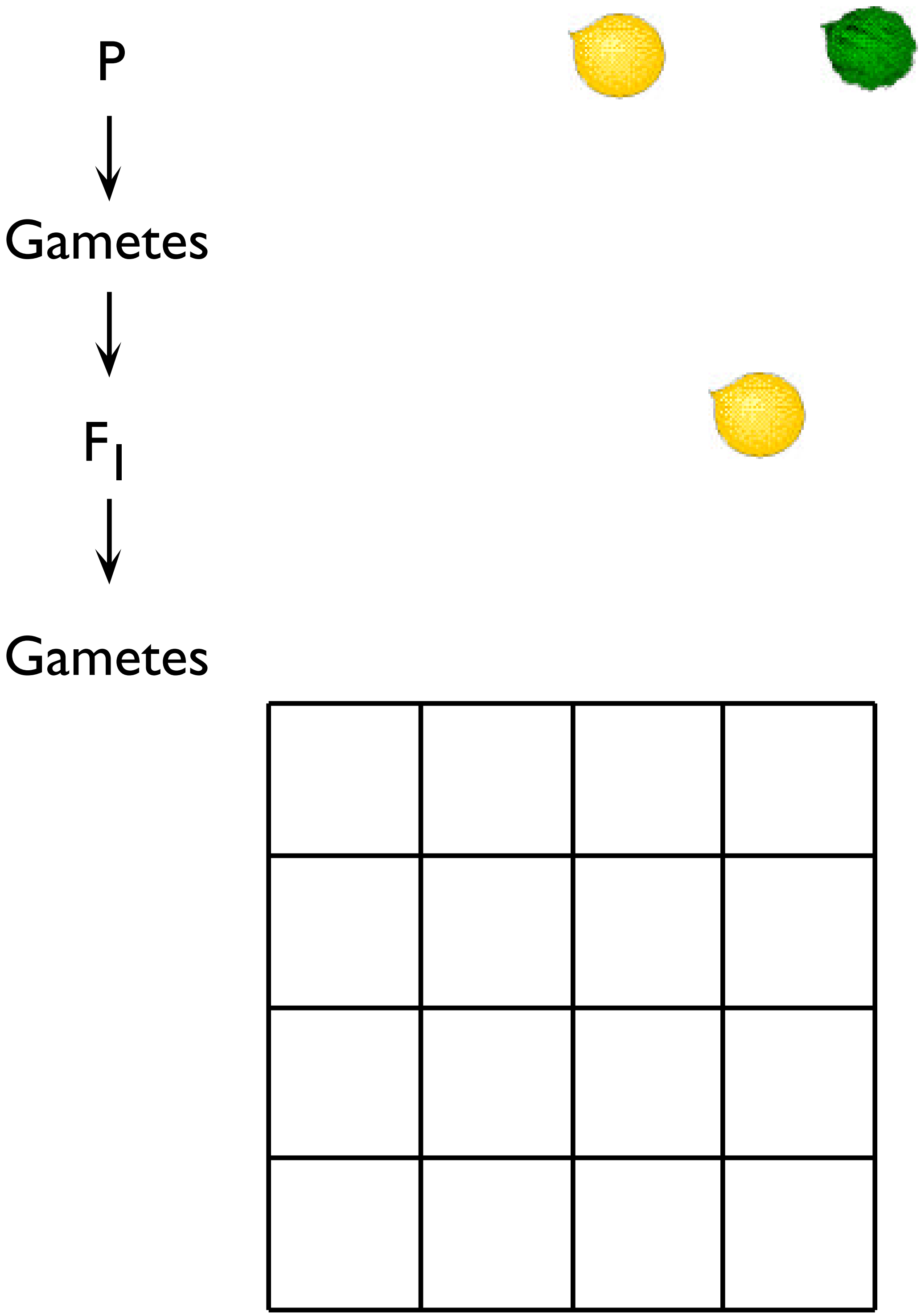


- As predicted for random segregation:



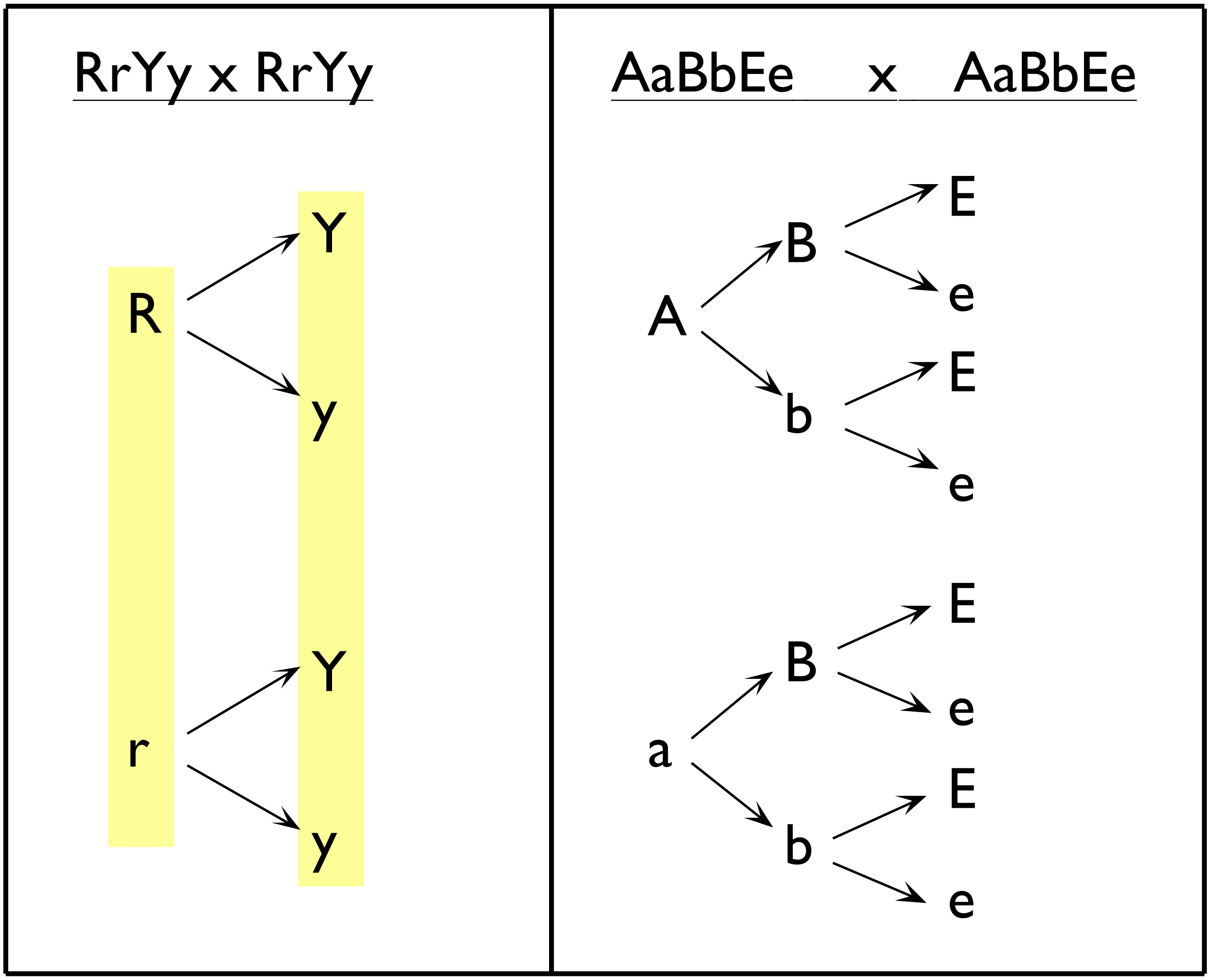
Therefore… expect that **segregation of determinants on different chromosomes** should be **independent of each other**

Mendel's experiments cont'd…



Segregation of alleles of one gene is independent of segregation at another gene — law of **independent assortment**

Branch diagrams – consider one phenotype at a time; overall ratio is product of individual ratios



Predicting the results of crosses…

For any multi-factor cross showing independent assortment –

- How many gamete classes?

- How many progeny phenotypes?

- How many progeny genotypes?

Need:

- to be able to predict genotype/ phenotypes ratios

- large sample sizes

- systematic way of evaluating whether the observed results are really different from the expected results

Probability

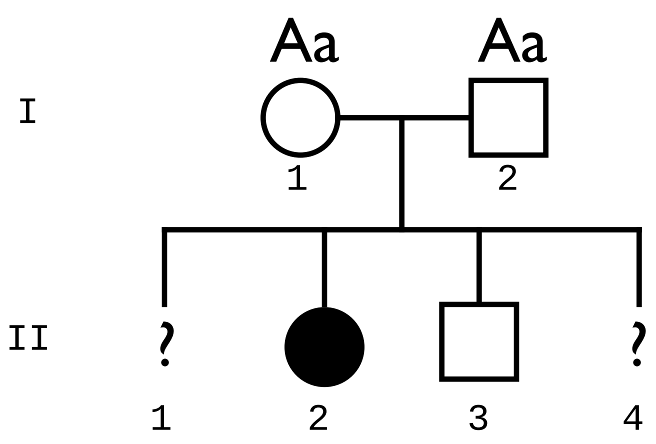
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Predicting outcomes

The goal: Estimating the chances of a particular outcome actually occurring

Why bother?

Consider this pedigree:



Is II-1 **female** or **male** ? How probable is each outcome?

Is II-4 **A_** or **aa**? How probable is each genotype?

Probability:

- of an **inevitable** event=
- of an **impossible** event=

If x, y, and z are the only possible outcomes of an event, P(x) + P(y) + P(z) =

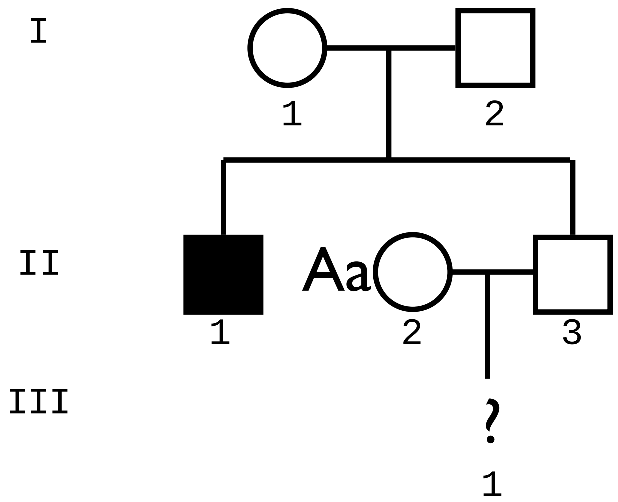
Imposing multiple conditions

Product rule

The probability that two or more **independent** events will occur (event *x* **and** event *y* **and** …)

Examples

What is the probability that III-1 will be **aa**?



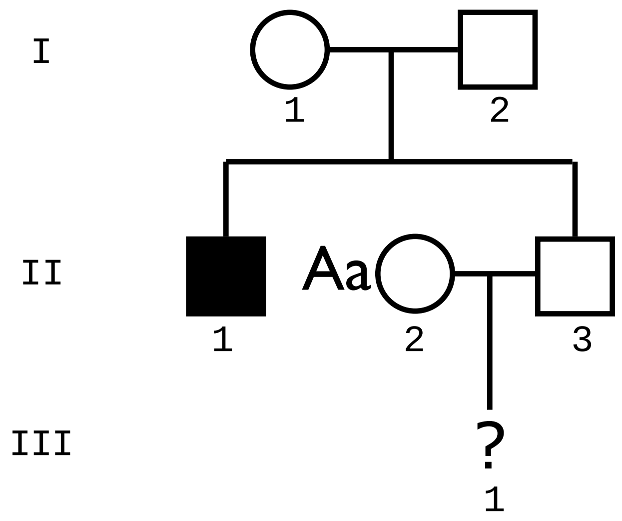
Relaxing the criteria

Sum rule

The probability of an outcome that can be achieved by more than one way (event *x* **or** event *y* **or**…)

- When you pick a card…probability that it is a **red 5** ?

- Probability that III-1 is **homozygous** ?



Probabilities of sets of outcomes

Binomial expansion

…to determine the probability of a specific set of outcomes in a number of trials that could each have either of two possible outcomes

e.g., determining the probability of 1 female and 4 male children in a family with 5 children

Equation: $(a + b)^5 = 1$

$a^5 + 5a^4b + 10a^3b^2 + 10a^2b^3 + 5ab^4 + b^5$

1. Find the term where the exponents match the numbers you want

2. Substitute the individual probabilities

fraction of 5-children families expected to have 1 daughter and 4 sons:

Evaluating results…

Assessing the **goodness of fit**

χ^2 **analysis** – How likely is it that the deviation from the predicted values is due to chance alone?

Null hypothesis – that there is no real difference between observed and predicted results

Example: flipping a coin to decide if it's a trick coin…

χ^2 analysis:

1. Compute χ^2 value:

$$\chi^2 = \sum \frac{(\text{observed - expected})^2}{\text{expected}}$$

2. Determine **df** (the # of degrees of freedom)

3. Look up P value in χ^2 table

Exercise:

Are the results of this Drosophila cross consistent with independent assortment of the two genes (sv^+ and spa^+)? Can you explain these results? [**Hint:** refer back to the chromosome theory of inheritance.]

$$\frac{sv^+}{sv}\ \frac{spa^+}{spa} \times \frac{sv}{sv}\ \frac{spa}{spa}$$

$$\downarrow$$

# of progeny	Phenotype of progeny
759	sv^+ spa^+
2	sv^+ spa
0	sv spa^+
770	sv spa

Remember that sv^+ and spa^+ are the dominant phenotypes; sv and spa are recessive.

Chi-square table

P ⇨ df	0.995	0.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005	⇦P df
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879	1
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597	2
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838	3
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860	4
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750	5
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548	6
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278	7
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955	8
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589	9
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188	10

Linkage and recombination

Explanation for the *Drosophila* cross (lecture 8 end):

…but how to explain the results of this Drosophila cross?

[pr = purple eyes; vg = vestigeal wings
Both are recessive alleles; "+" alleles are wildtype]

pr^+ pr sc^+ sc x pr pr sc sc

↓

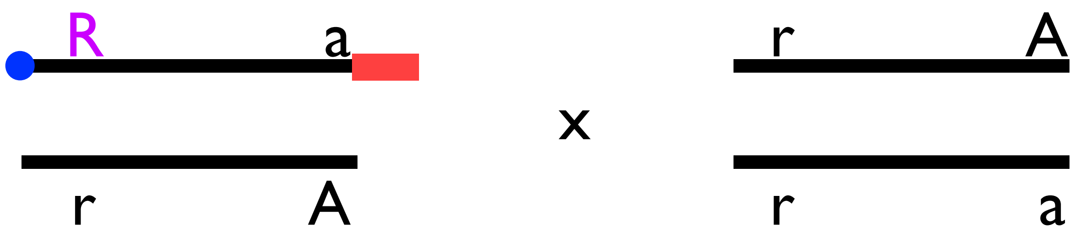
pr^+ sc^+	pr^+ sc	pr sc^+	pr sc
1339	151	154	1195

Morgan's explanation, based on cytology of meiosis–
recombinant class arising from crossover

How to test? What's needed?

Harriet Creighton & Barbara McClintock, maize
Curt Stern, Drosophila

Experimental setup:



R= colored endosperm
r = colorless
A = starchy endosperm
a = waxy

Note the two salient features that make this experiment feasible:
"knob" and translocation –
genetic markers –

Look for **colorless, waxy** progeny

Ask: what do the chromosomes look like in these progeny?

Their results:

Importance of crossovers?

- proper segregation of homologs

- new combinations of alleles

Mapping genes



Aa Bb x aa bb		Aa Dd x aa dd		Aa Ee x aa ee	
500	AB	420	AD	350	AE
20	Ab	60	Ad	120	Ae
20	aB	60	aD	120	aE
500	ab	430	ad	350	ae

Can you deduce the map order of these genes?

Insight from Alfred Sturtevant (1913)—

If recombination sites are random,

- probability of recombination between a pair of genes…

- recombination probability in adjacent intervals…

⇒ **Recombination frequency** can be used as a measure of **genetic map distance**

1 map unit = 1 **centiMorgan** = 1% of meiotic products being recombinant

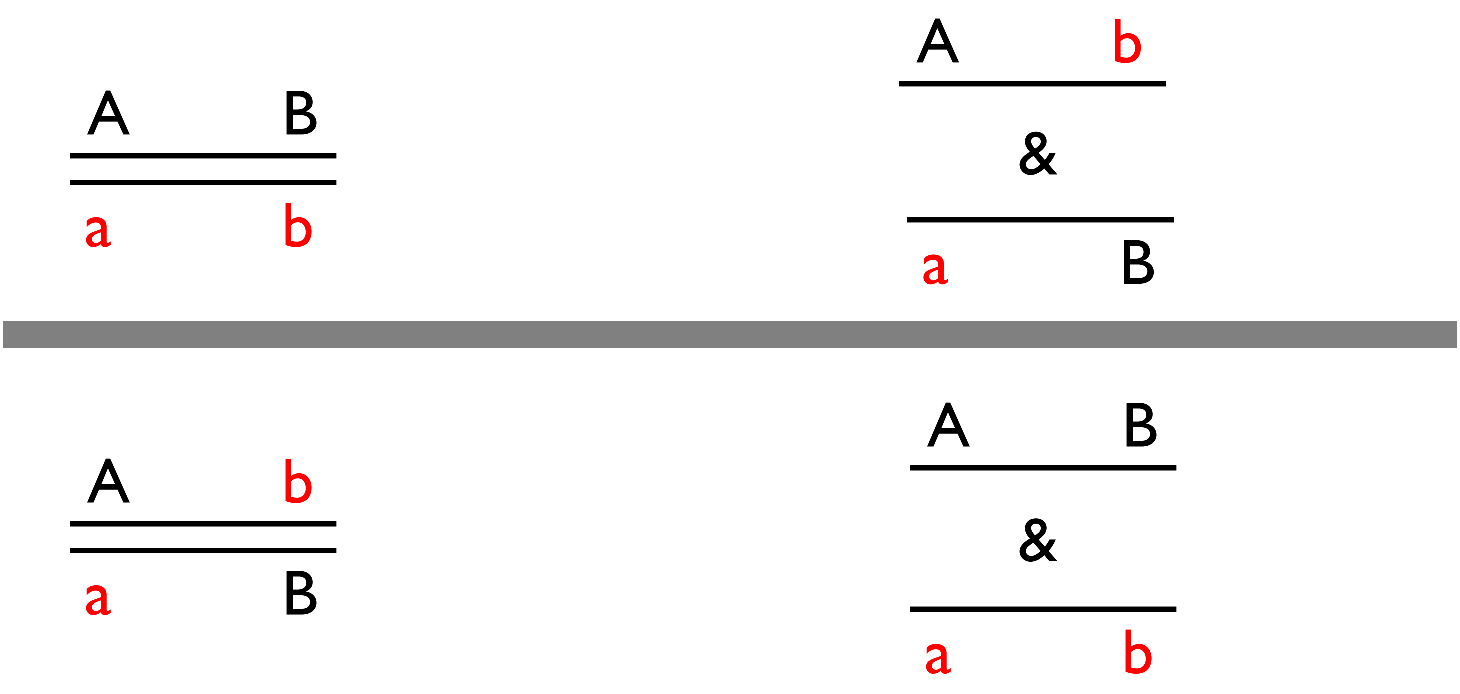
Constructing genetic maps

1. Are the loci linked? (What is a **locus** anyway?)

2. How much recombination?

How do we identify the recombinant gamete classes?

Parent | **Recombinant gametes***



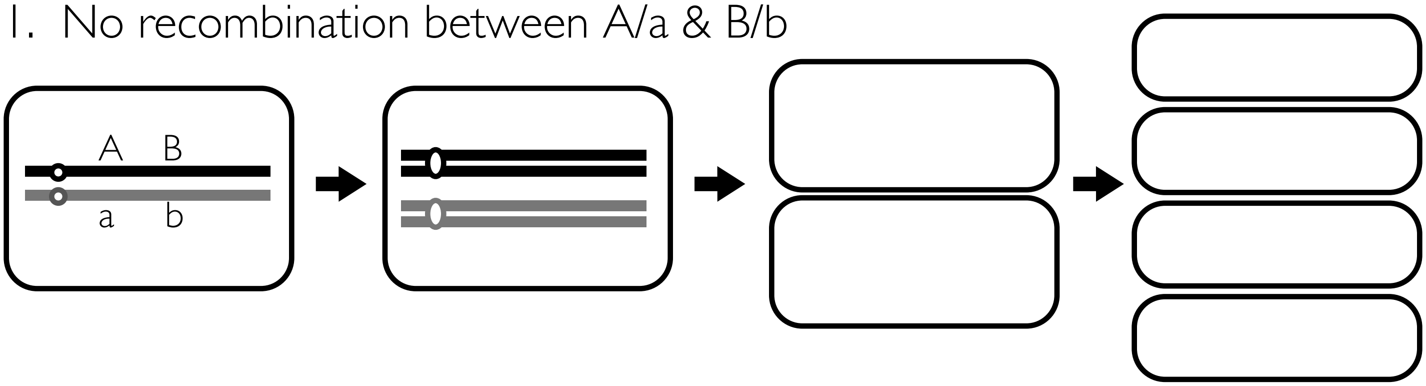
*Fill out the worksheet to be sure you understand this

Operational definition for “non-parental”:

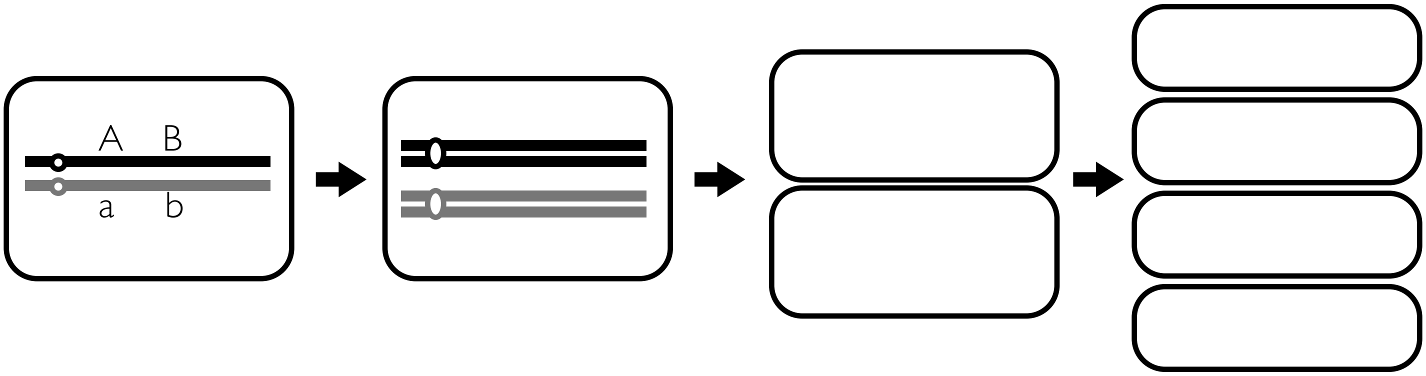
Generally, the cross is
heterozygote x homozygous recessive
…why?

Meiosis worksheet

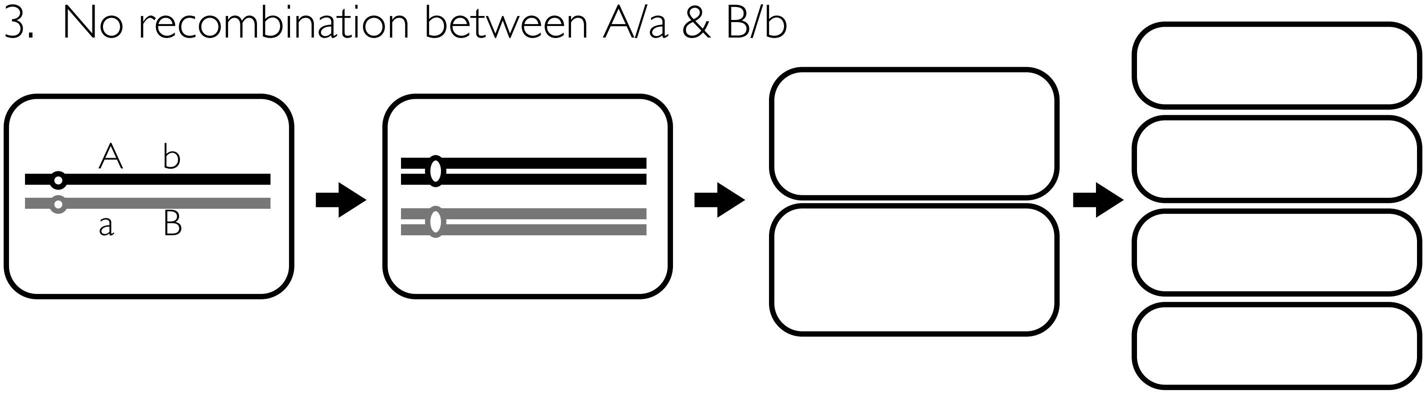
1. No recombination between A/a & B/b



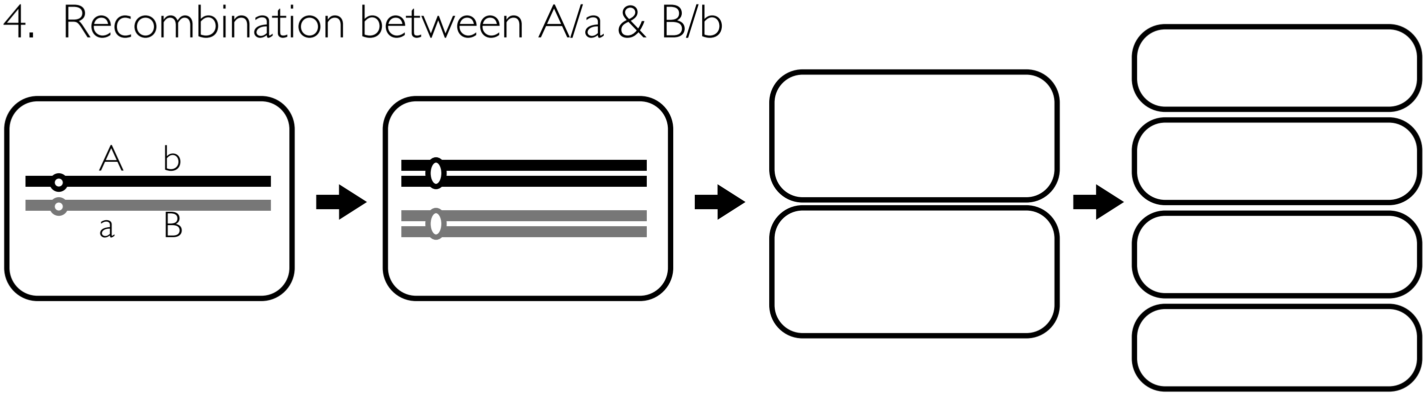
2. Recombination between A/a & B/b



3. No recombination between A/a & B/b



4. Recombination between A/a & B/b



Gene mapping - I: Three-point test cross

What is the maximum recombination frequency in any interval?

The range of possibilities:

Tightly linked

↓

Independent assortment

Unlinked loci:

Loci can appear to be unlinked because:

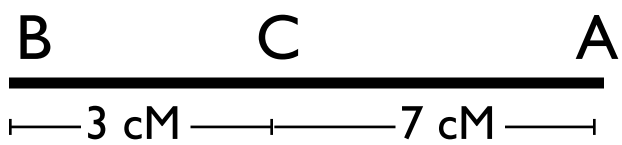
3-point test cross – what is it; why do it?

Requirements for successful 3-point test cross:

- Triply heterozygous strain (producer of recombinant gametes)
- A cross that will reveal the genotypes of the gametes…

Example 1. Predict the progeny phenotypes and numbers for this cross

Parent 1: $\dfrac{+\ \ +\ \ a}{b\ \ c\ \ +}$



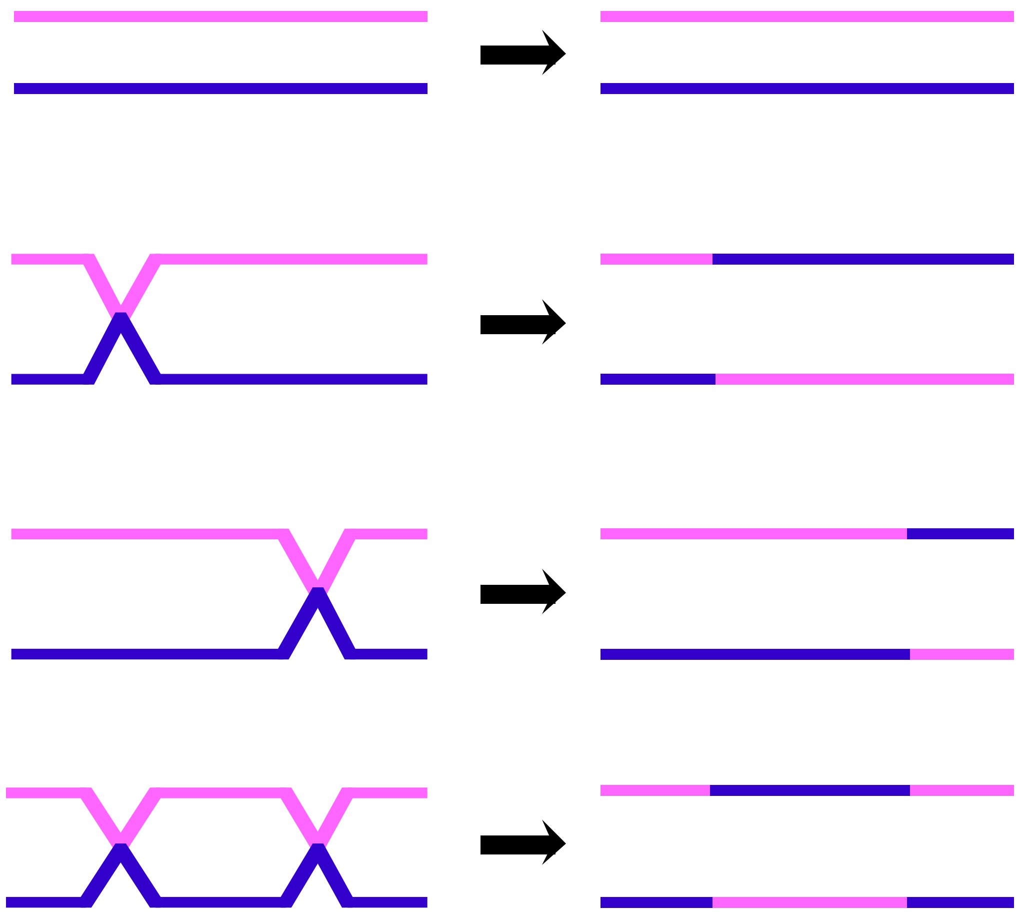
Parent 2: $\dfrac{b\ \ c\ \ a}{b\ \ c\ \ a}$

…count 10000 progeny

Step 1. Determine the phenotype and number of the double-crossover (DCO) products

Step 2. Determine the phenotype and number of the single-crossover (SCO) products

Step 3. Determine the number of the parental (non-crossover, NCO) types



Example 2. Construct a linkage map (order and distance) for the following genes

Genes:	cu (curled wing)
	sr (stripe body)
	st (scarlet eye)

Parents:

Female:	cn/+	rd/+	vg/+
Male:	cn/cn	rd/rd	vg/vg

Progeny phenotypes:

cn vg	4202
rd	4258
cn rd	28
vg	32
cn rd vg	264
+ + +	276
rd vg	482
cn	458

Step 1. Identify the parental, SCO and DCO classes

Step 2. Determine the gene order—

Knowing the allele composition of the parental class, what gene order could generate the observed DCO classes? (Trial and error!)

Step 3. Add up the recombination frequencies to obtain the map distances

Genetic maps may not correspond directly to physical maps

What could cause the genetic map to deviate from the physical map?

- Map expansion:
- Map contraction:

Interference and **coefficient of coincidence**

Genomics

Genetic maps in humans

The trouble with humans…

- Markers

- Crosses

Establishing linkage: which chromosome?

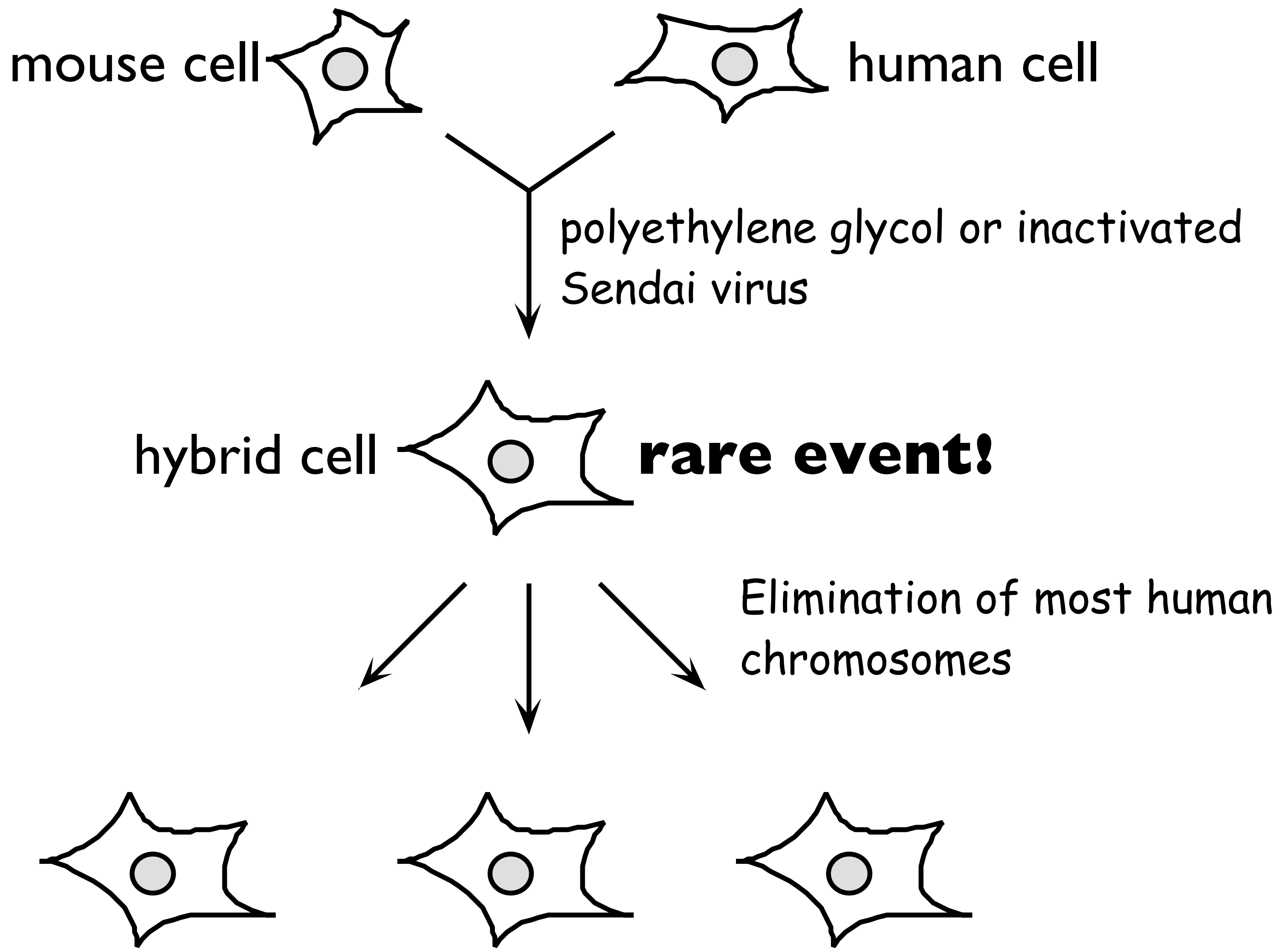
Recognizing chromosomes

- ◇ Size

- ◇ Staining pattern – bands



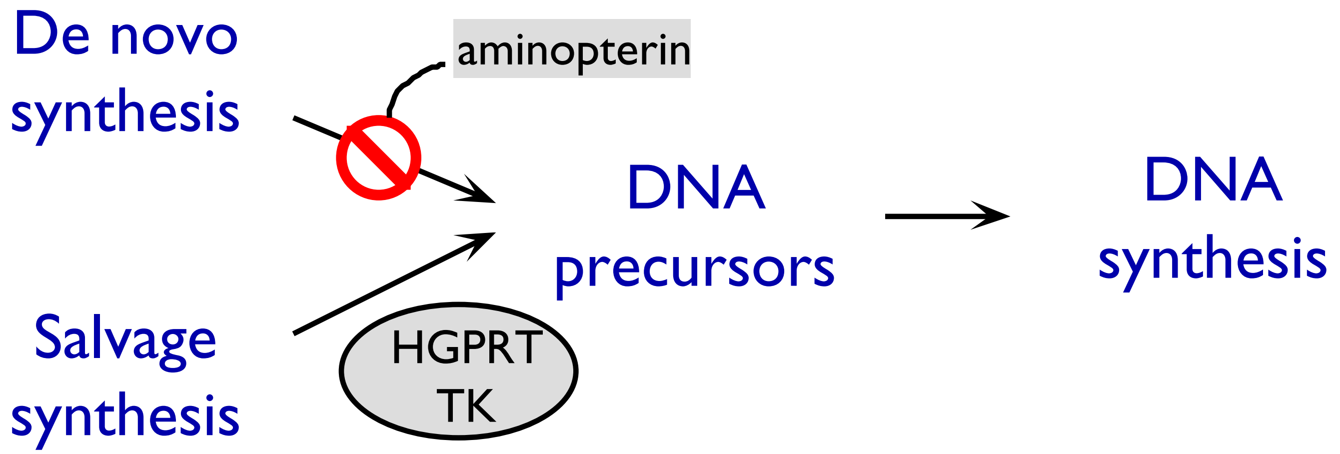
Somatic cell hybrids



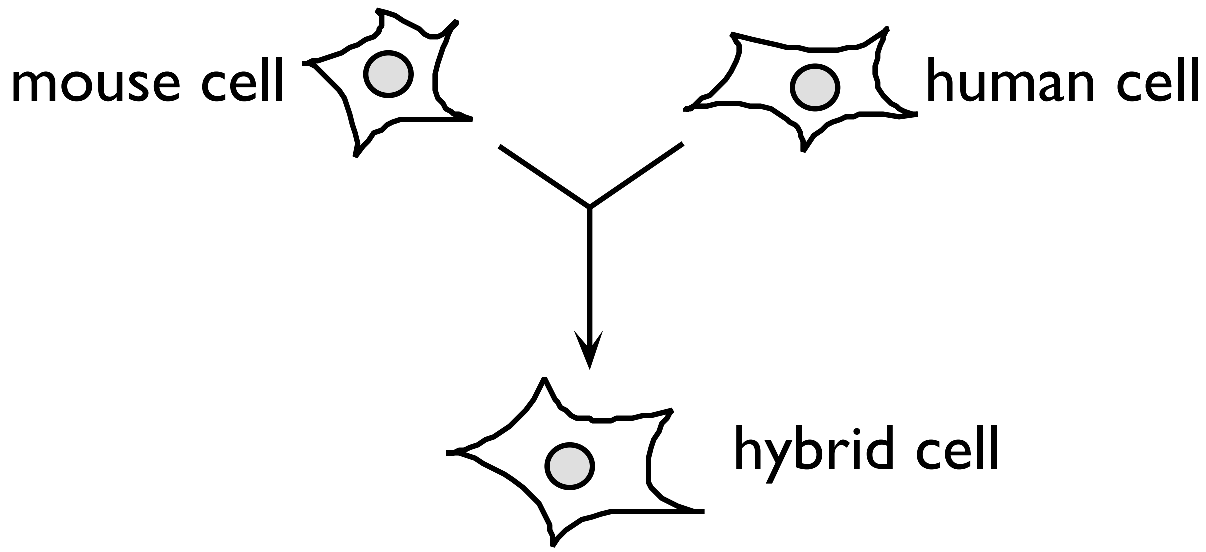
Hybrid cell lines: mostly mouse plus a few human chromosomes

How to pick out those rare fusion events?
…**selection** based on DNA precursor synthesis

Two pathways of DNA precursor synthesis:

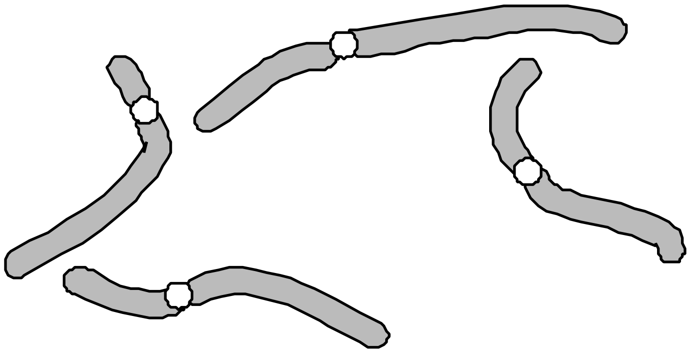


To select fusion product…



Fluorescent in situ hybridization (FISH)

Hybridize fluorescent-labeled probe to chromosome spread



…can be used in combination with somatic cell hybrids

Mapping by linkage

…linkage with respect to what?

The conventional approach – look at recombination frequency between the gene of interest and a neighboring marker gene

Conventional markers (alleles that result in overt phenotypes) are hard to come by…

But DNA sequence differences (polymorphisms) are plentiful

Therefore: construct a map of polymorphic sites

To map a gene: look at recombination frequency between the gene of interest and a neighboring polymorphic site

…so, we use DNA sequence polymorphism as just another pair of alleles – without an overt phenotype, but detectable

Useful polymorphisms

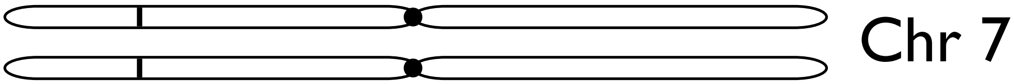
Originally – Restriction fragment length polymorphisms (RFLP)

These days – **Sequence repeat** polymorphisms

Repeated sequences constitute up to 35% of the human genome

- **Minisatellite repeats:** ~ 30 bp
- **Microsatellite repeats:** ~2–5 bp
  - Dispersed throughout the genome
  - Highly variable numbers of repeats at each location; individuals often heterozygous

What's polymorphic about microsatellite repeats?



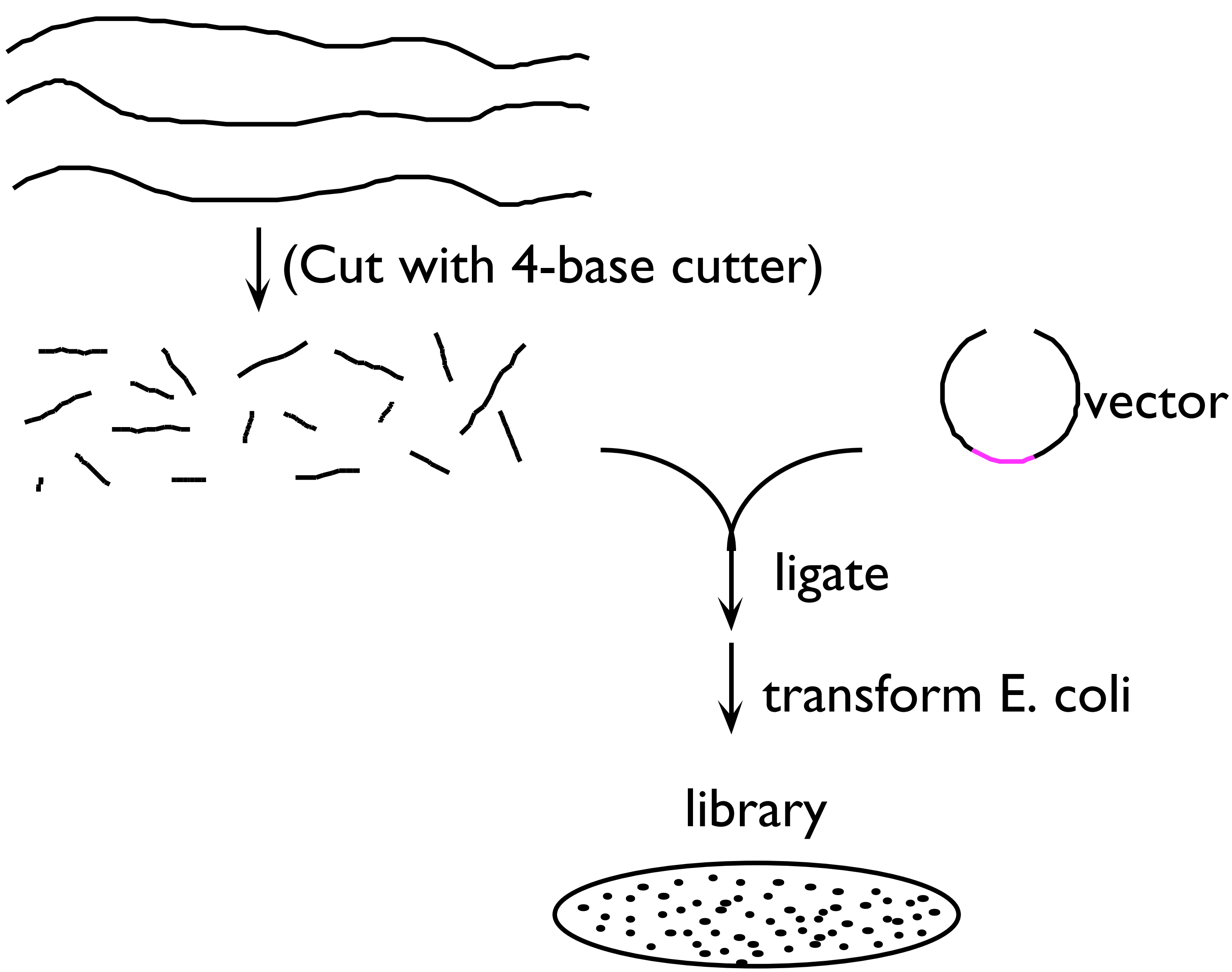
Person 1
- 21 repeats on chromosome 7 homolog 1
- 33 repeats on chromosome 7 homolog 2

Person 2
- 30 repeats on chromosome 7 homolog 1
- 18 repeats on chromosome 7 homolog 2

The advantage of microsatellite repeats:

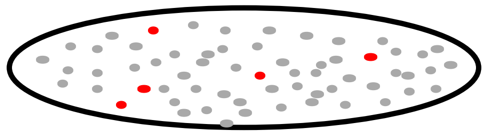
Map construction: Identifying repeats and their genomic locations

Step 1. Make genomic **library** of short inserts



Step 2. Identify repeat-containing clones

Synthesize DNA for probe: → probe the library
e.g., $(CA)_{20}$



identify positives

↓

sequence the inserts

Clone 1

...TTACACCGAACACGCCAAGAGAAACACACACA
CACACACACACACACACAATACGGTTTCGGTGGTTA
ATTTAGCT...

Clone 2

...TAATTTAATTTTAATTGGGTTTCACACACA
CACACACACACACACACACACACACACACACACA
CACAGTTTGATTATTGCTACTTAC...

etc.

Step 3. Identify chromosomal locations of the repeat sequences

e.g., by hybridization to metaphase chromosomes (somatic cell hybrids come in handy!)

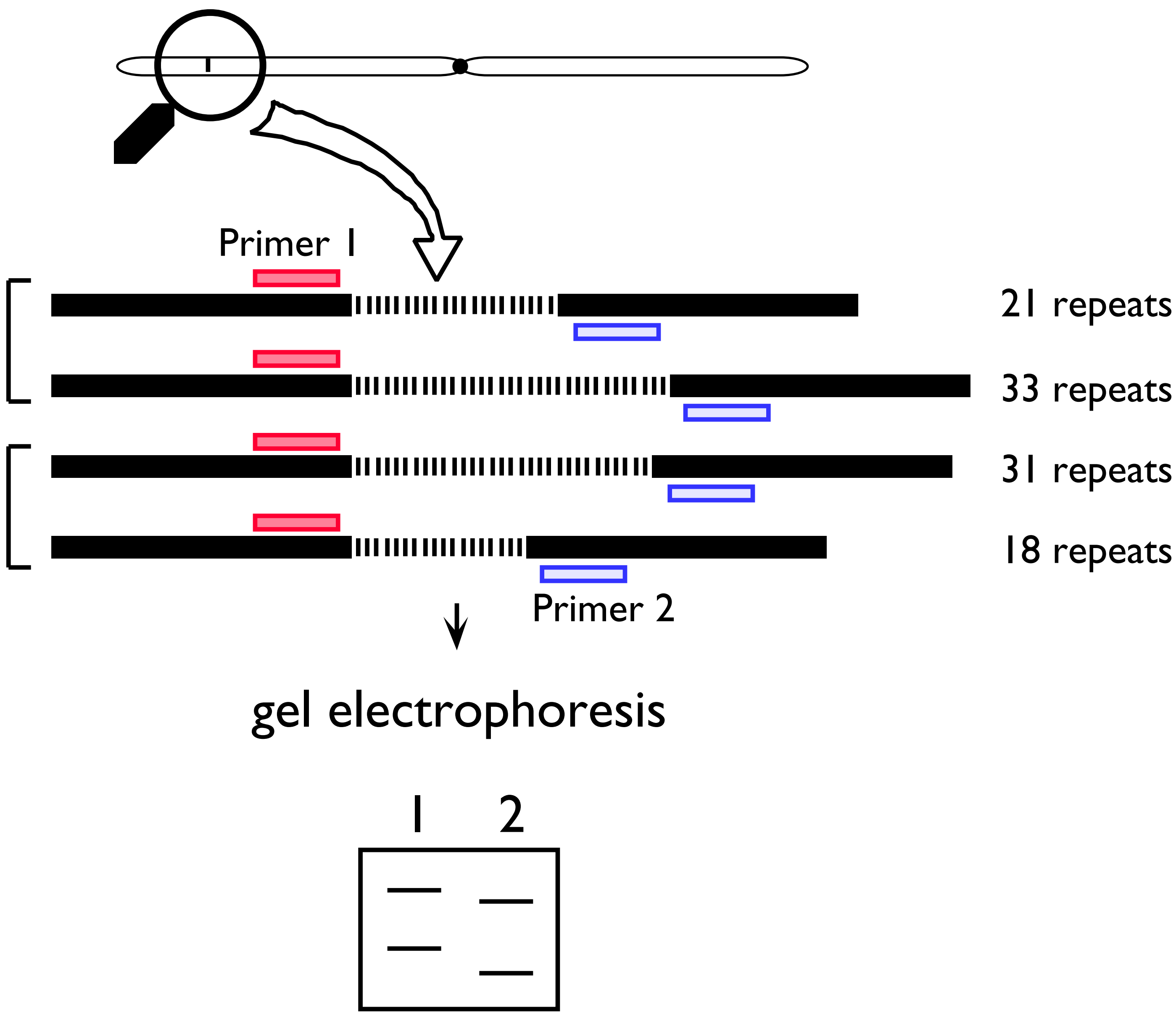
Step 4. Constructing a profile: How many alleles in the population? How frequent?

Usually done by **Polymerase Chain Reaction** (PCR)

Determining repeat number at a polymorphic locus...

- PCR using unique sequence (flanking the repeat) as primers

Using our chromosome 7 example again:



Using polymorphisms to map disease genes

- Score disease gene allele based on overt phenotype
- Score polymorphic alleles based on PCR analysis
- Ask: can recombinants be detected?

In practice:

- Obtain DNA sample from all family members (blood ⇨ tissue culture)
- For each individual:
  - score disease phenotype, determine genotype
  - score polymorphism on each homolog (e.g., 21,33) for each of many polymorphisms
- For each polymorphism, calculate **Lod score** for various map distances

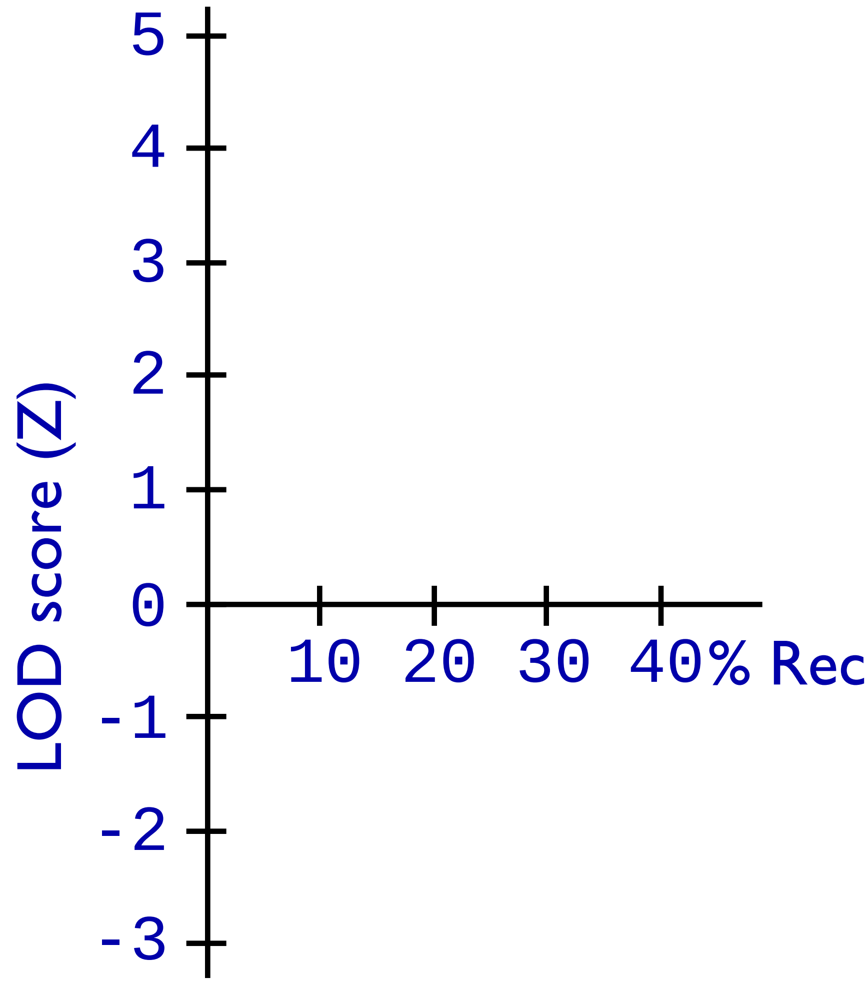
Lod score = log of odds of linkage

$$= \log_{10}\left[\frac{\text{likelihood of linkage}}{\text{likelihood of not being linked}}\right]$$

Computing LOD scores –

- Take a pairwise combination of disease gene and a polymorphic locus…
- Ask:
  - What's the probability of getting this pedigree if the two loci are **linked**…
  - What's the probability of getting this pedigree if the two loci are **unlinked**?
- Calculate LOD score
- Repeat

A hypothetical example –



Lod score of 3 = 95% probability of linkage at the proposed recombination frequency

From Lod scores – sites with highest probability of linkage to the gene

Lod scores from different pedigrees can be **added up**!
Why?

Linkage to marker sites – can be starting point for cloning the gene… **Positional cloning**

Not trivial – 1–2 cM…still ~1–2 million bp to search!

Approaches to cloning the gene

- "brute force"

- **Candidate gene** approach

- **rescue** of disease phenotype in a model system

Other applications of polymorphic site mapping technology

- **Diagnostics**

- **DNA profiling/genetic fingerprinting**

  Tabulate allele frequencies for various polymorphic sites – e.g.,

  Polymorphic site 1: 20 alleles (21-40 repeats), equal frequencies

  Polymorphic site 2: 30-40 repeats:

i.	0.15	(30 repeats)	vii.	0.05	(36 repeats)
ii.	0.12	(31 repeats)	viii.	0.10	(37 repeats)
iii.	0.08	(32 repeats)	ix.	0.09	(38 repeats)
iv.	0.09	(33 repeats)	x.	0.13	(39 repeats)
v.	0.06	(34 repeats)	xi.	0.08	(40 repeats)
vi.	0.07	(35 repeats)	xii.	0.08	(all others)

What is the probability of a person having alleles ii and iv of polymorphic site 1, and alleles v and ix of polymorphic site 2?

Some applications of DNA profiling

- Forensics
- Paternity
- Conservation biology

Mutations and mutagenesis

What is a mutation?

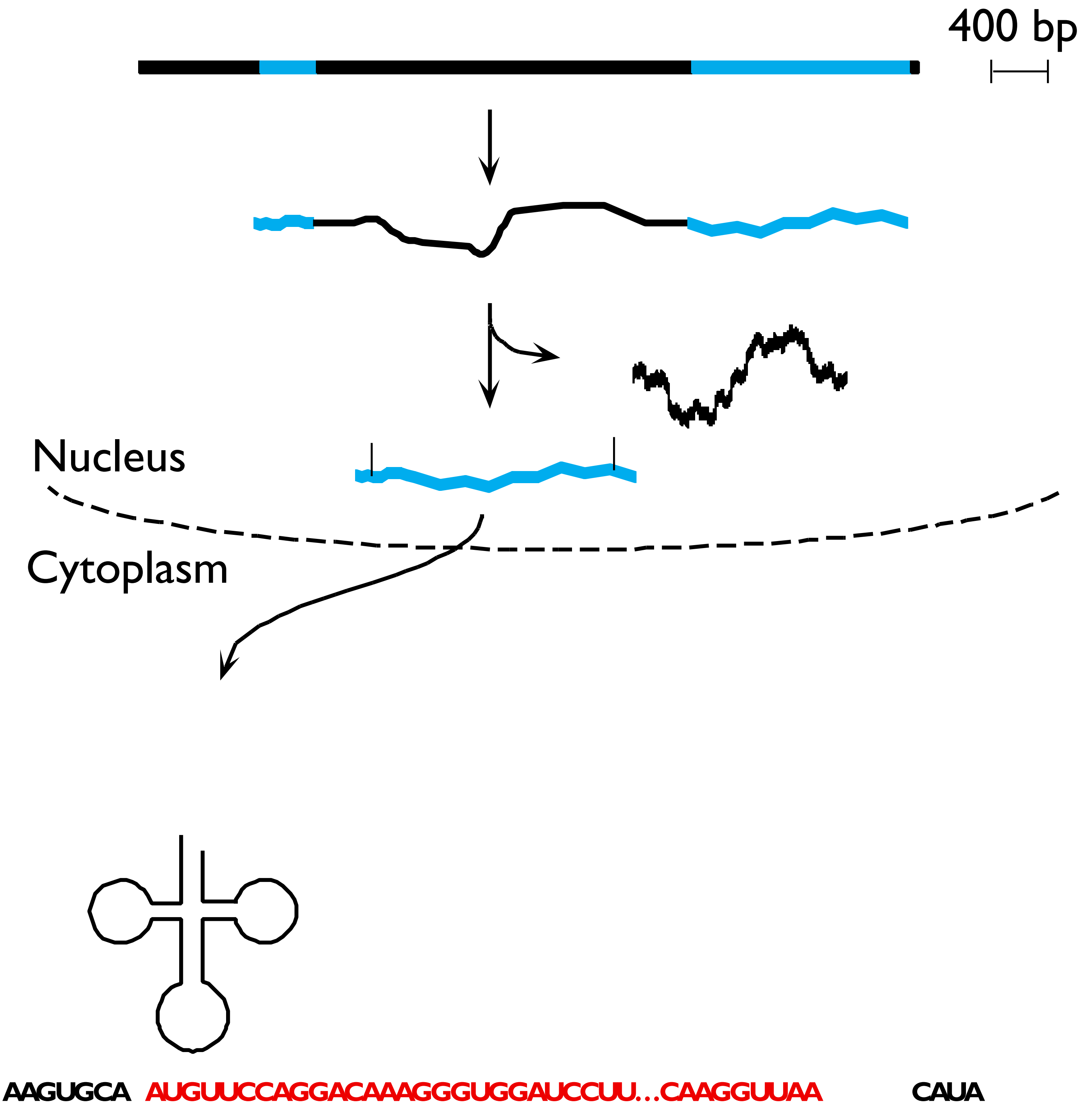
- **Chromosome** mutations

- **Point mutations**
  - Base substitutions
  - Insertions/deletions—
    frameshift mutations

Is a mutation an allele?

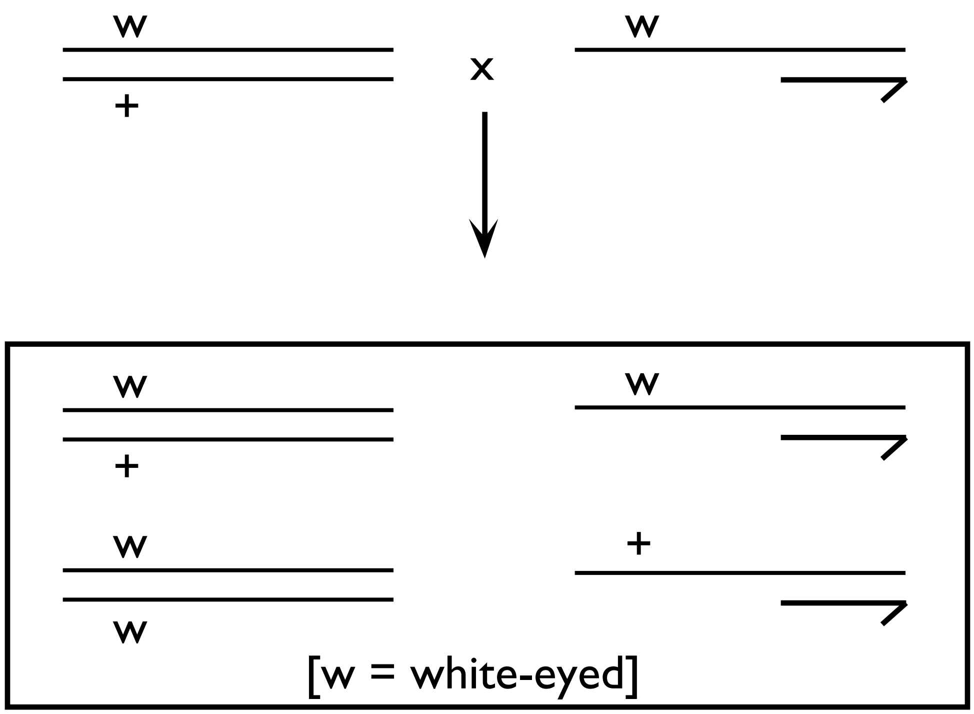
Where can things go wrong?

Drosophila **yellow** gene



Mutation frequency

H. J. Muller's assay – **How frequently** does the *Drosophila* X chromosome acquire mutations?



Asked…what fraction of crosses **failed** to give red-eyed male progeny?

Conclusion: ~2 mutations per 1000 X chromosomes

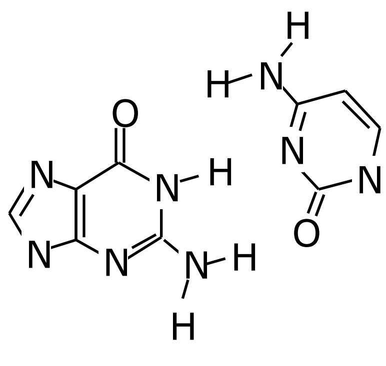
Extrapolating to humans…

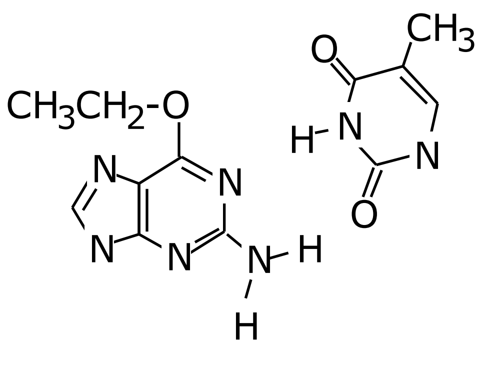
Inbreeding, and why it's not a great idea

Some causes of mutations

- **Misincorporation** during replication

- **External causes**
  - Radiation
  - Chemical mutagens – e.g.:
    - Alkylating agents





    - Intercalating agents

Damage control

- **Preventing** misincorporation –
- Normal activities of polymerase:
  - Extension of 3' base-paired primer
  - Removal of 3' unpaired base
- If incorrect base is put in…

Correcting misincorporation –
Mismatch repair:

1. Identify mismatched bases
2. Identify the original (parental) strand
3. Correct the other strand

Timeout for repair – **Checkpoints**

Lee Hartwell and Ted Weinert, UW (1989)



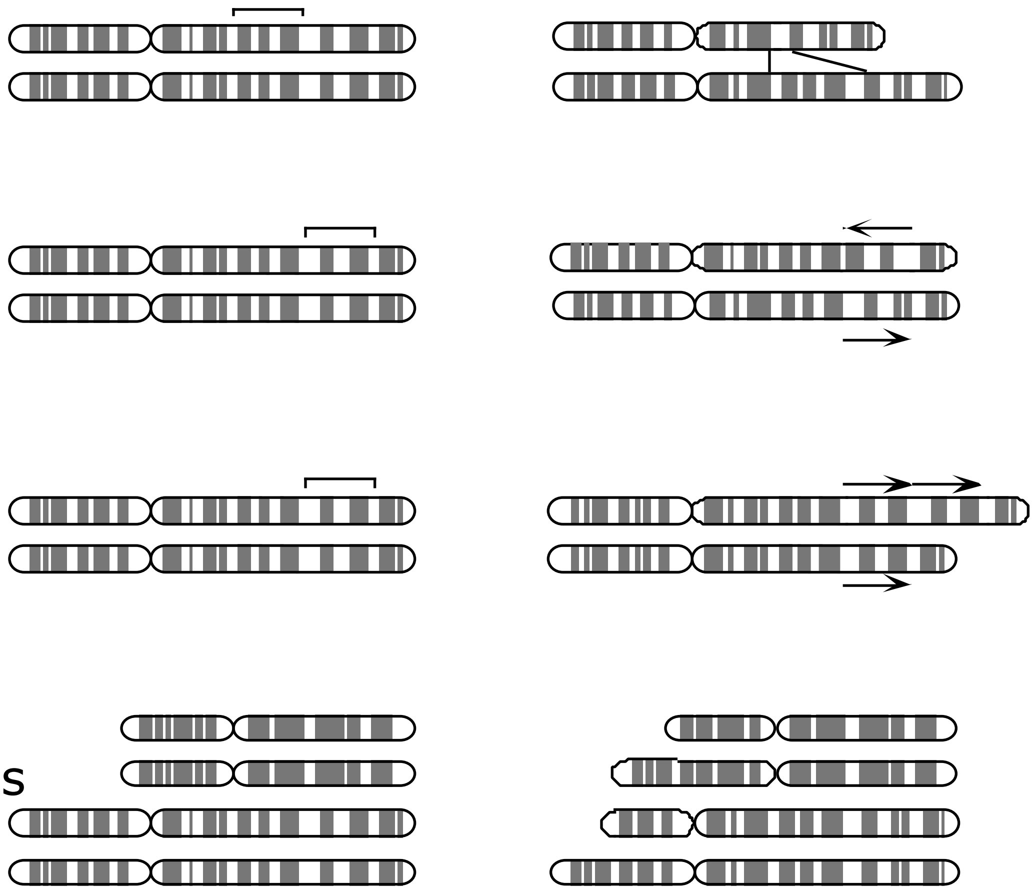
Phenotype of mutant?

Chromosomal abnormalities

- Changes in chromosome number

- Changes in chromosome structure

  - Deletions
  - Inversions
  - Duplications
  - Translocations



What's the tolerance limit for "gene imbalance" ?

Deletions

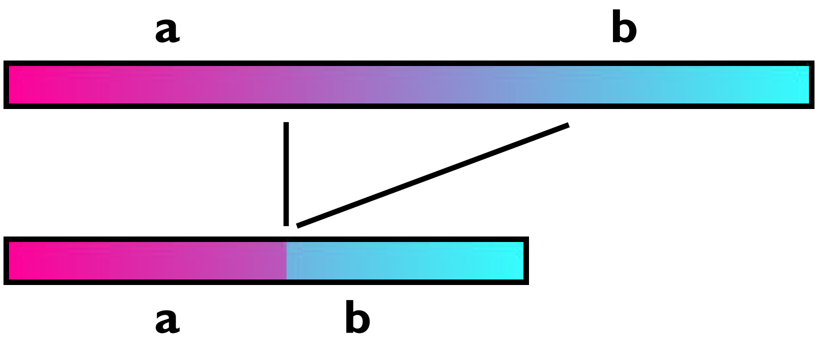
- Terminal vs interstitial



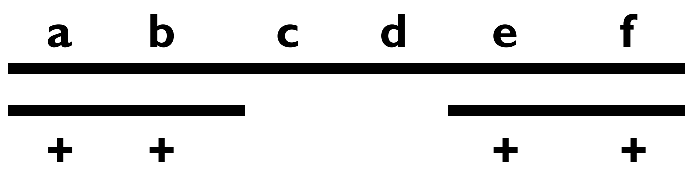
  - ◇ "cri du chat" syndrome in humans – terminal deletion in chr 5

- How are these deletion chromosomes transmitted?

- **Genetic consequences**
  - ◇ Reduced recombination frequency between markers flanking the deletion

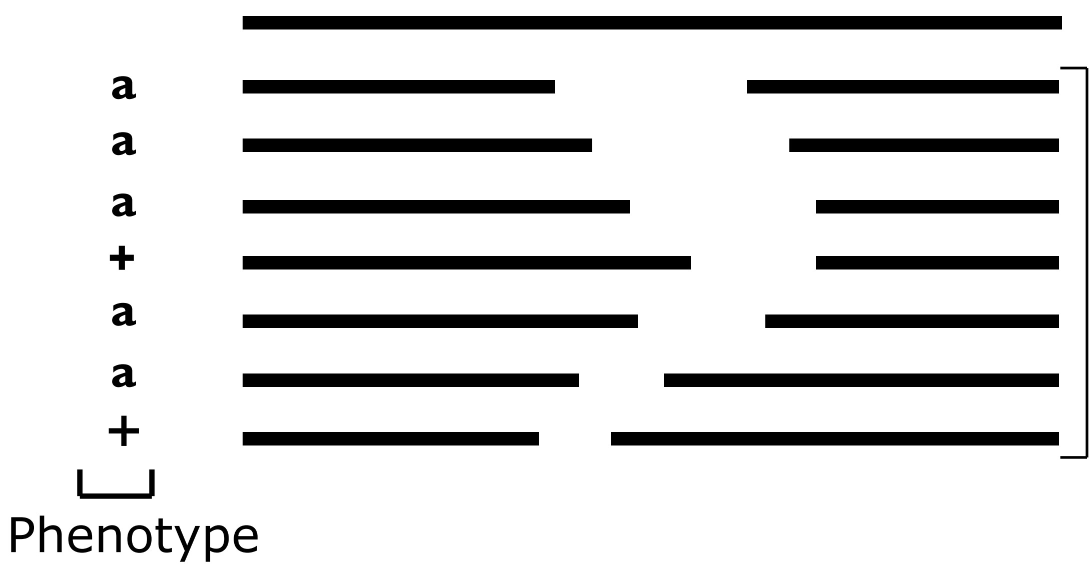


◇ Recessive alleles **uncovered**



Practical use: **deletion mapping** to locate genes

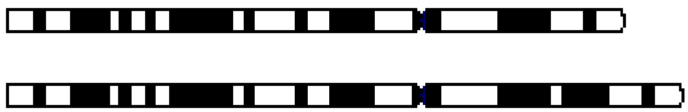
Set up crosses such that the progeny have the recessive allele of interest on one homolog and a deletion on the other… ask: which deletion uncovers the recessive allele?



Duplications

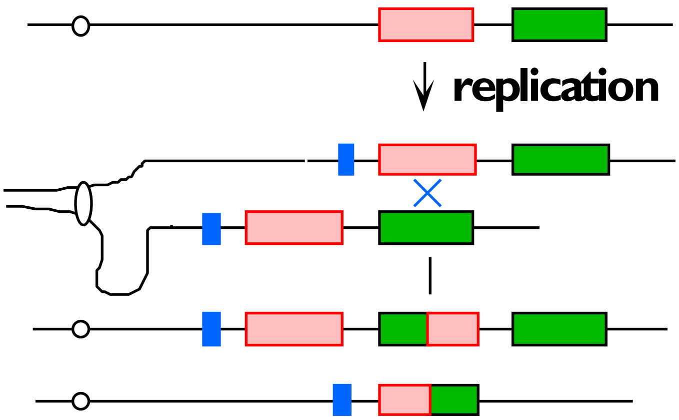
- Large-scale – e.g.,

  Charcot-Marie-Tooth syndrome



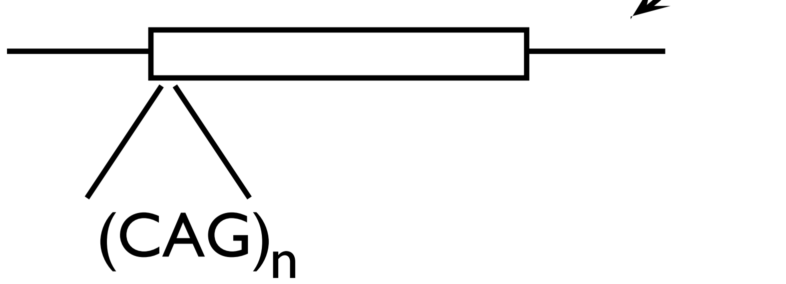
chr 17

- Microscopic/submicroscopic
  Can be caused by **unequal sister chromatid exchange** – e.g., one form of red-green color blindness



- **Trinucleotide repeat expansion**

e.g., Huntington disease
Fragile X syndrome
Myotonic dystrophy



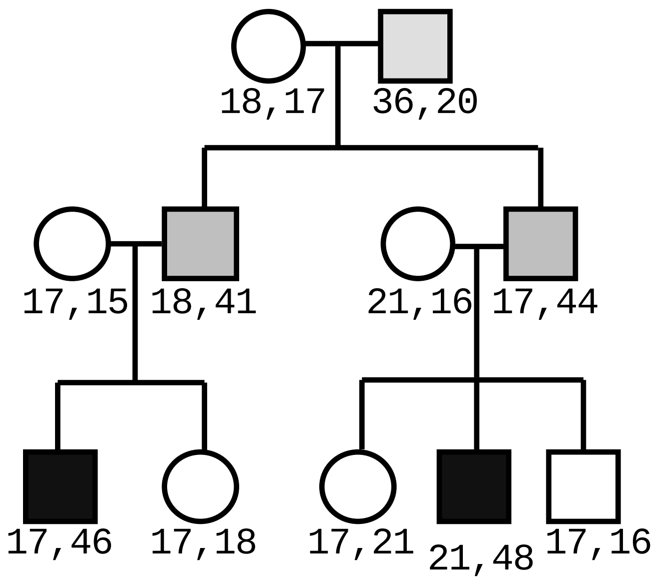
For Huntington…Normal n = 9 - 30

30 – 35 = "premutation"
≥36 : disease

Age of onset a number of repeats

Repeat #	Age of onset
≤40	42-84
41	30-66
42	35-59
43	23-61
44-45	22-54
46-49	21-48
50-55	20-44
≥56	7-23

"Anticipation" – progressively earlier onset



Mechanism of disease?

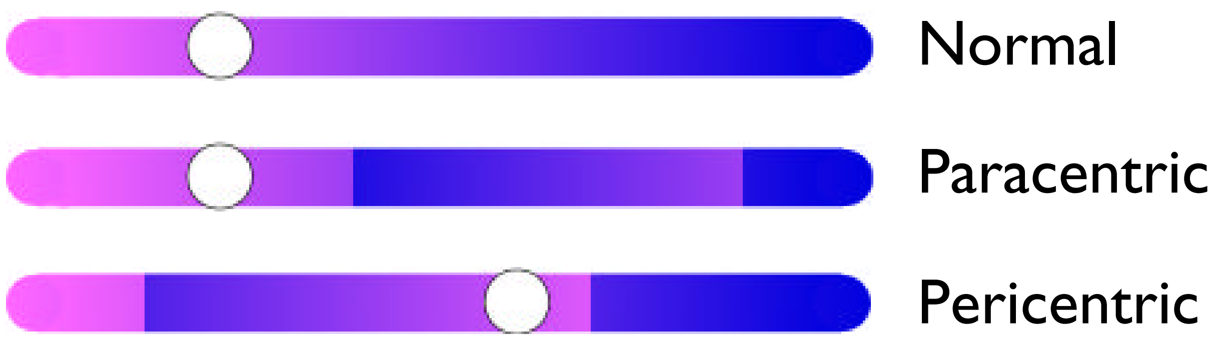
Mechanism of expansion?

Inversions

- From two internal breaks

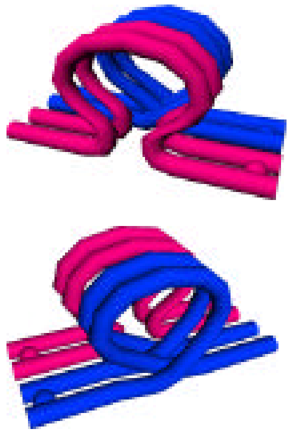
- **Phenotypes?**
  - Often no overt phenotype
  - Initial detection often on genetic grounds

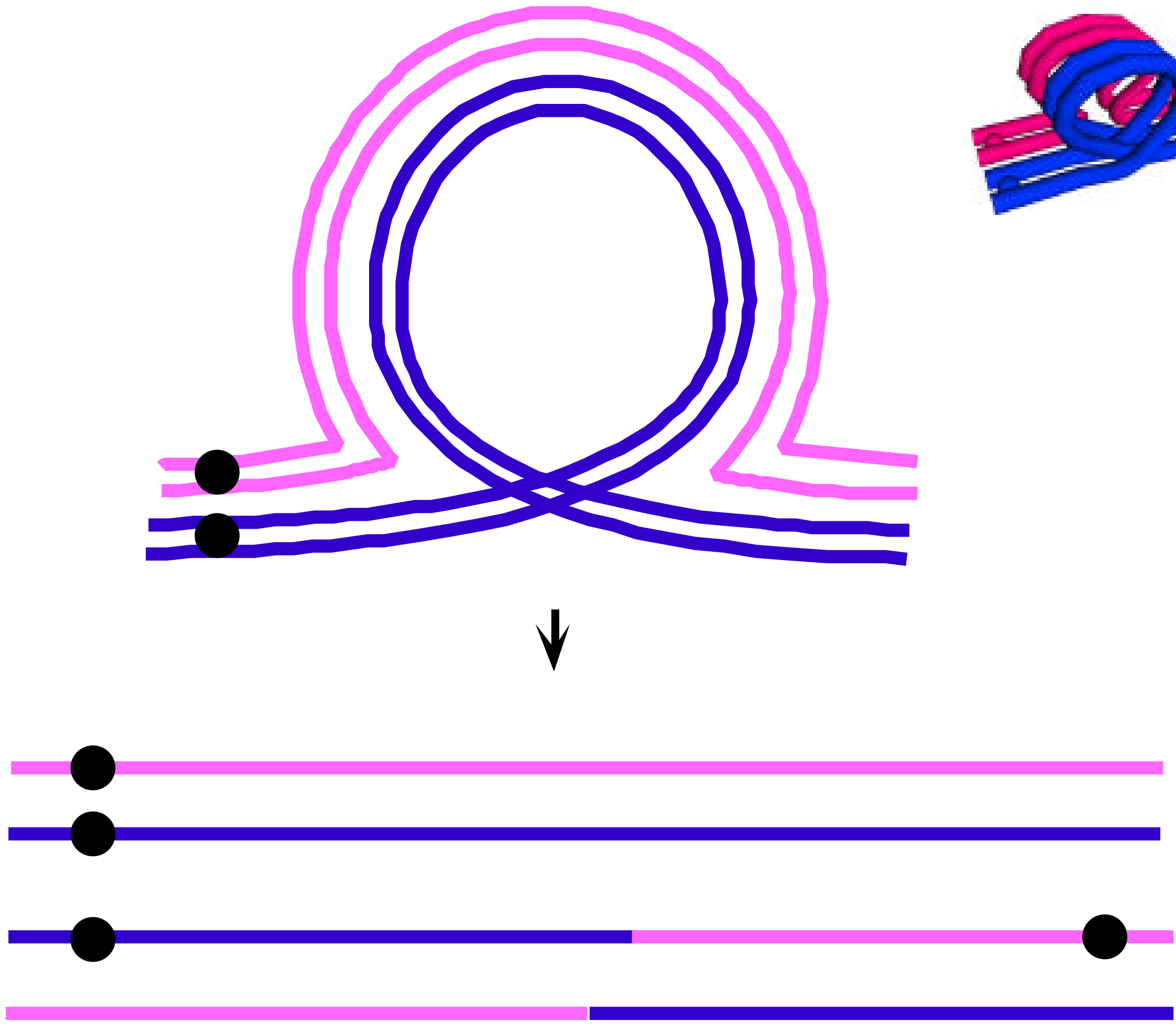
Paracentric and pericentric inversions



Meiosis and crossing over in inversion heterozygotes

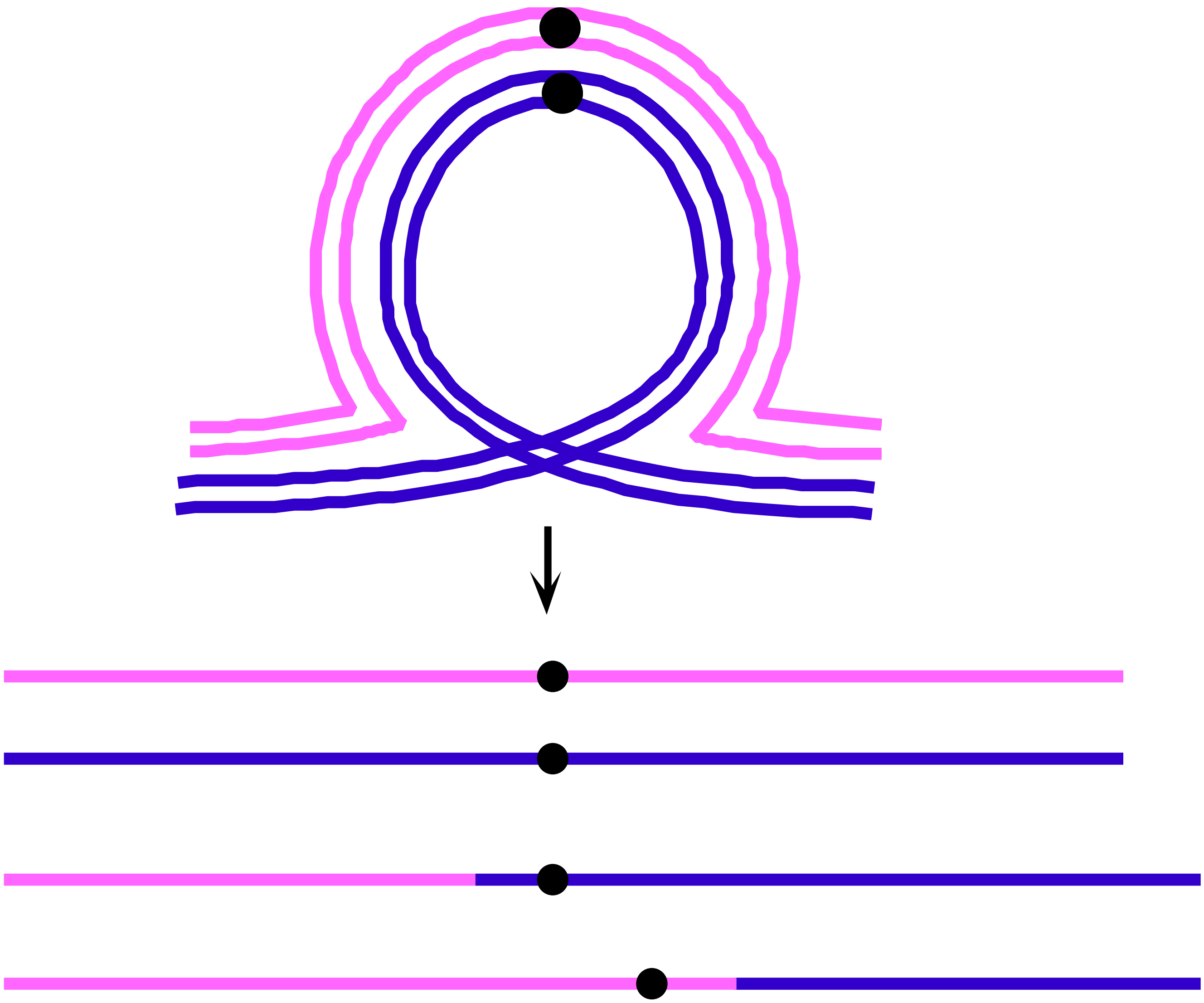
- Markers on the homologs are no longer co-linear…
  - **Paracentric inversions**





- Consequences?

- **Pericentric inversions**



- Consequences?

Translocations

- Often reciprocal
- Double heterozygotes can be viable

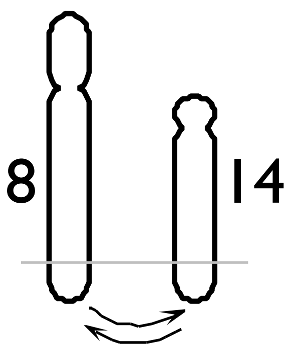
Phenotypes

– can cause some serious human disorders

- Associated with specific forms of **cancer**

  e.g., Burkitt lymphoma

  - one partner: chromosome 8
  - other partner: chromosome 14, 22, or 2

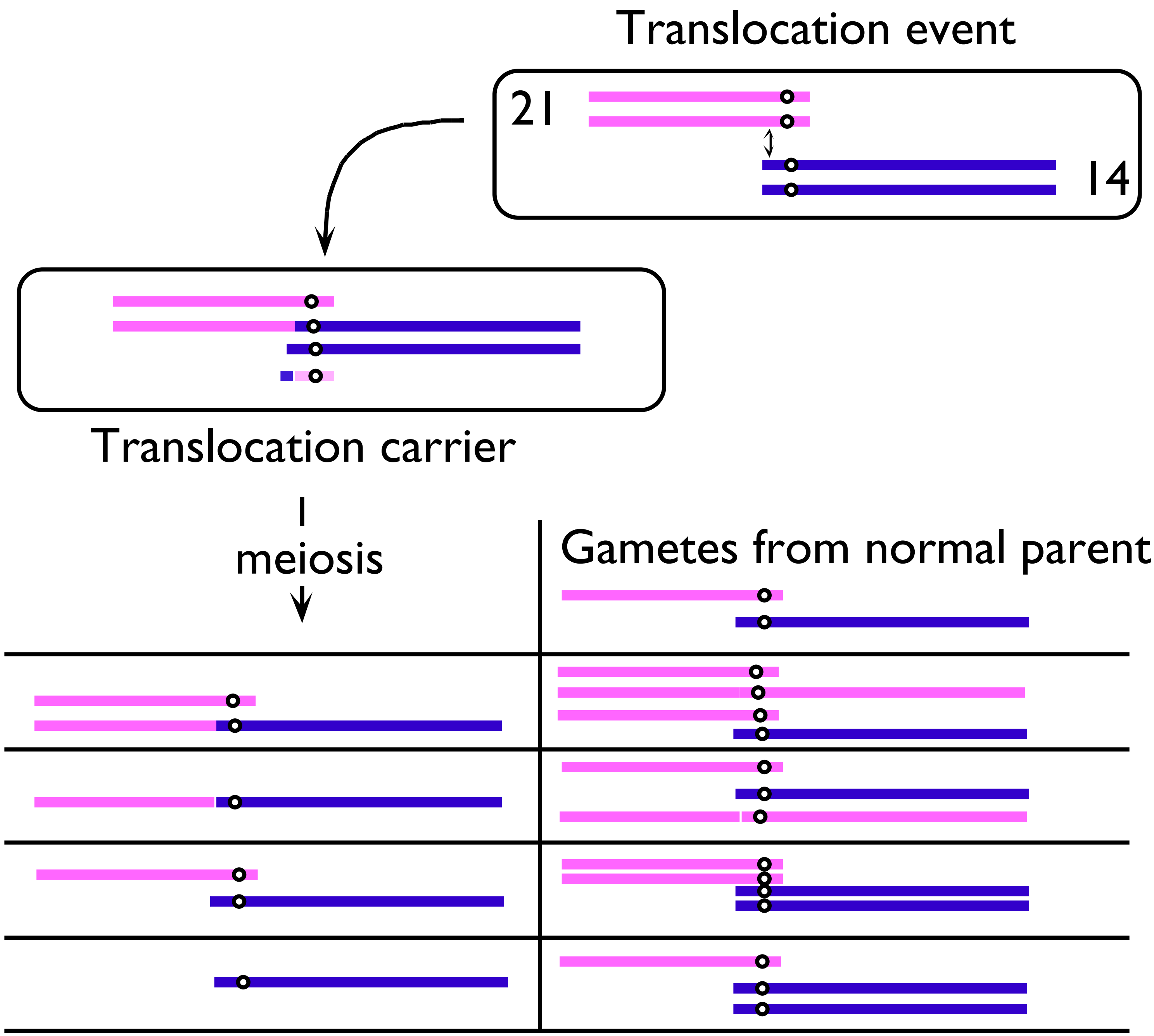


- **Non-cancer disorders**

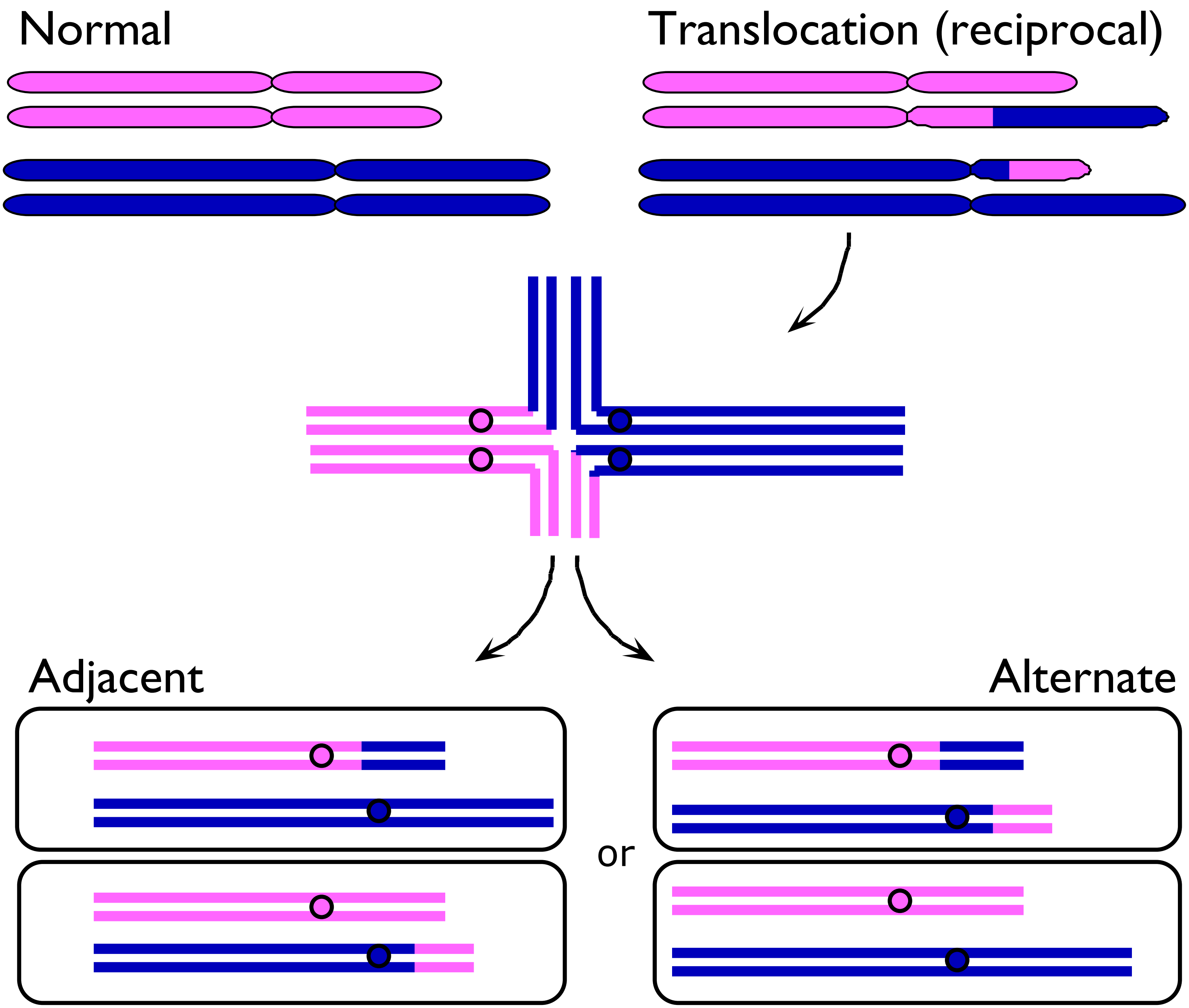
  e.g., translocation Down syndrome

  - **Robertsonian translocation** between chr 14 and 21

    long arms of two acrocentric chromosomes fused



Pairing and meiosis in double heterozygotes



Consequences

- Semisterility

Euploid: normal chromosome sets

Aneuploid: incomplete (unbalanced) chromosome sets

- In humans—aneuploidy in up to 35% of spontaneous abortions (6–20 weeks)

  - Monosomy: 2n - 1

    Human (females) — only one kind of monosomy…

    1 in 20000 live births

- Trisomy: 2n + 1

  Most common (at conception ?)— chr 16
  Most common at live birth— trisomy 21 —Down syndrome

  1 in 750 live births

- Less common:
  - trisomy 18 (1 in 10000)
  - trisomy 13 (1 in 20000)

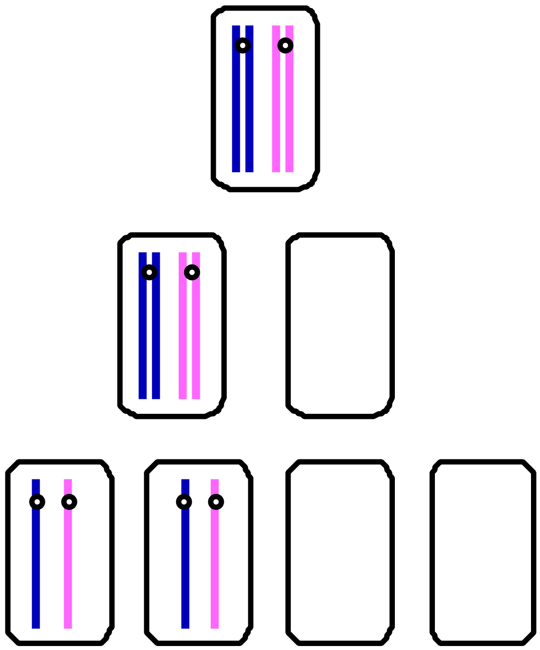
- Why better survival with trisomy 21 than other trisomies?

Hierarchy of tolerance of aneuploidy

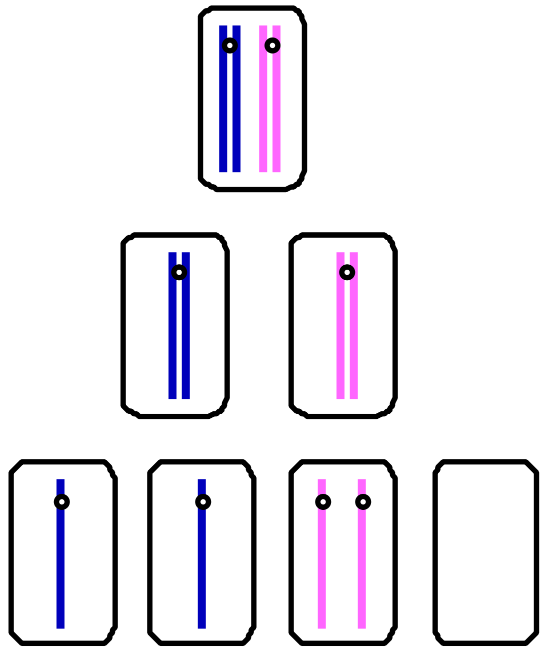
- sex chromosome aneuploidy > autosomal aneuploidy;
- autosomal triploidy > monosomy

Major cause of aneuploidy:
nondisjunction during meiosis

…can occur at Meiosis I



… or at Meiosis II

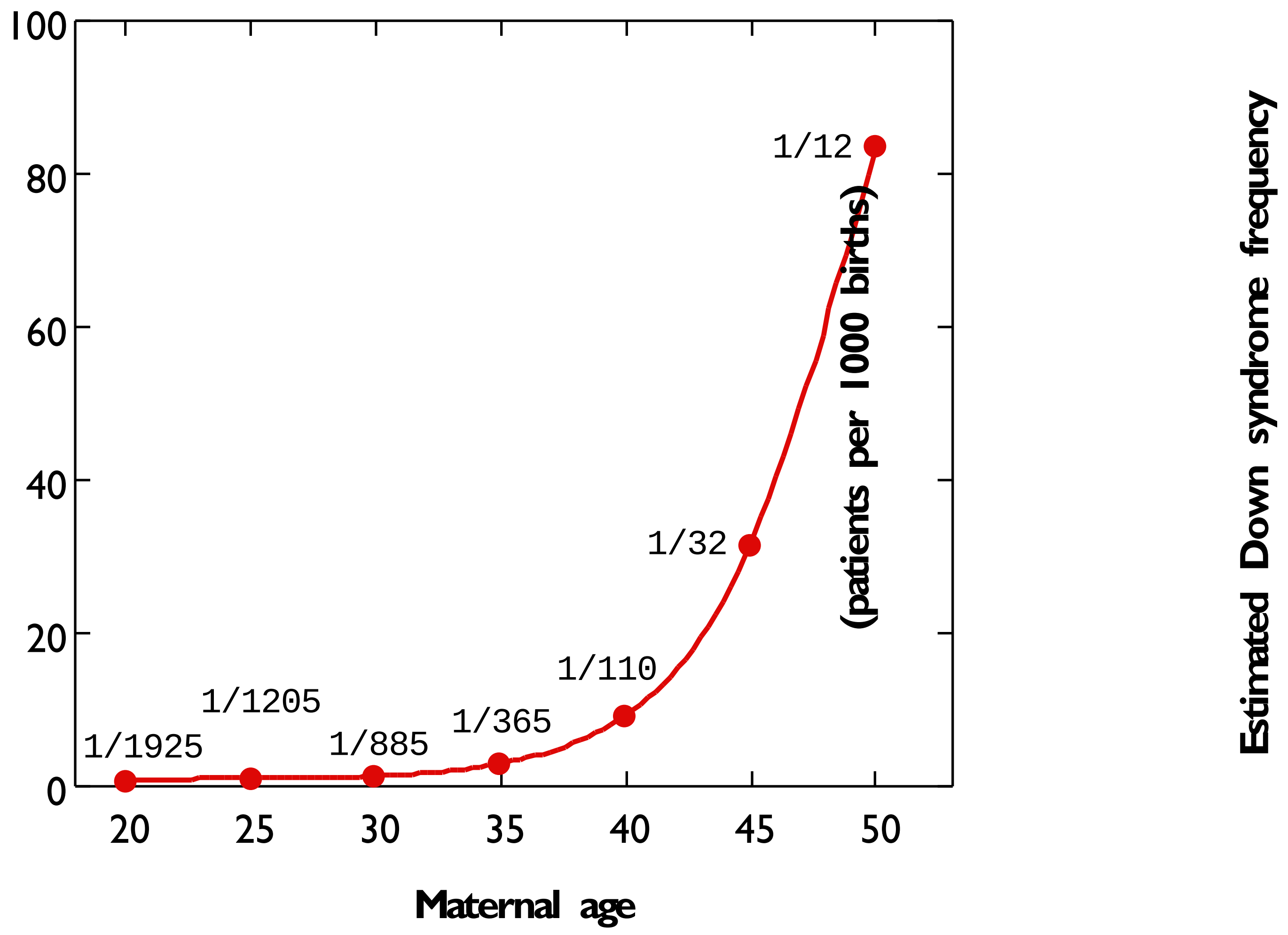


Consequences:

- Defective products

- Allele composition

Aneuploidy and maternal age



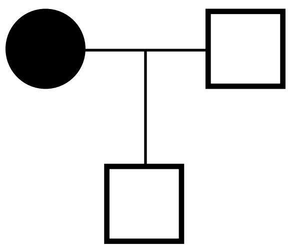
Why?

- ND↑ in older oocytes? Checkpoints?
- less robust spindle?
- increasing pool of "poor" oocytes?

About 20–25% of Down syndrome cases – **paternal** nondisjunction

Aneuploidy from **maternal** or **paternal** nondisjunction? Sometimes, clues from the pedigree…

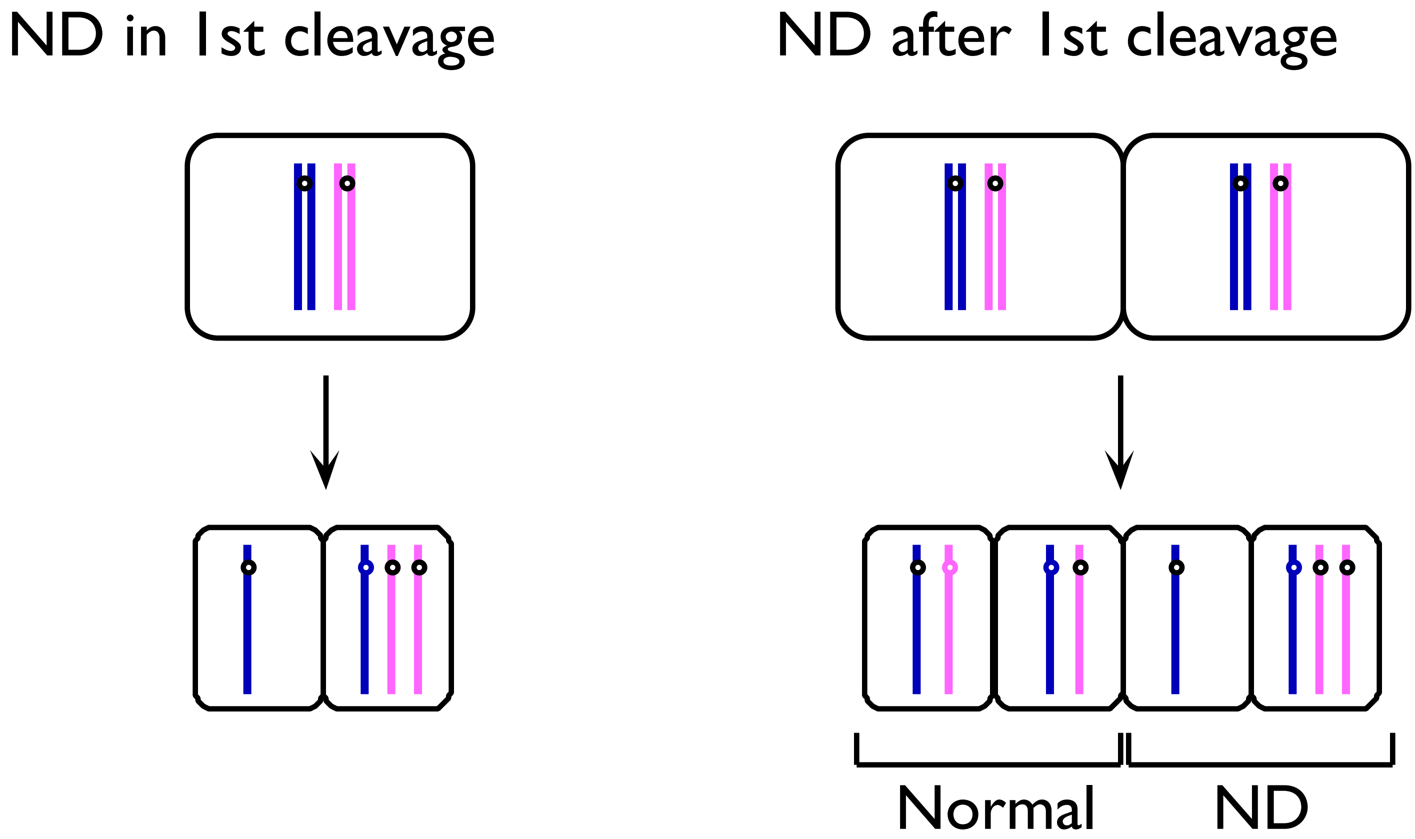
X^g = X-linked recessive condition
Paternal or maternal ND here?



Klinefelter (XXY) male

Mitotic nondisjunction

e.g., Down syndrome mosaics



Ploidy changes

- **Plants:** It's not all bad news… polyploidy is often desirable
  - Polyploids larger
  - Infertility due to polyploidy
- **Animals:** Haploids, polyploids rare

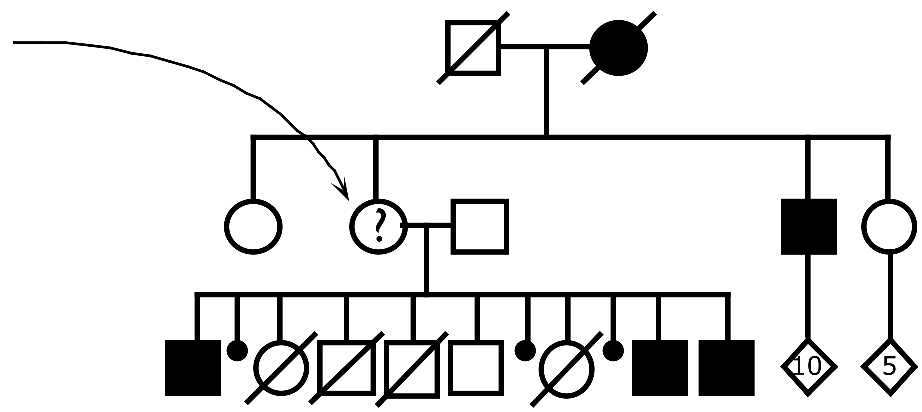
  Triploidy in humans –

Puzzling behavior of X-linked traits

- Dosage: Viability is extremely sensitive to gene dosage…so how to explain **XX** vs. **XY**?

- **"Exceptional females":** X-linked traits not showing the phenotype expected for the genotype – e.g., Becker-type muscular dystrophy, X-linked recessive

Genotype of II-2?



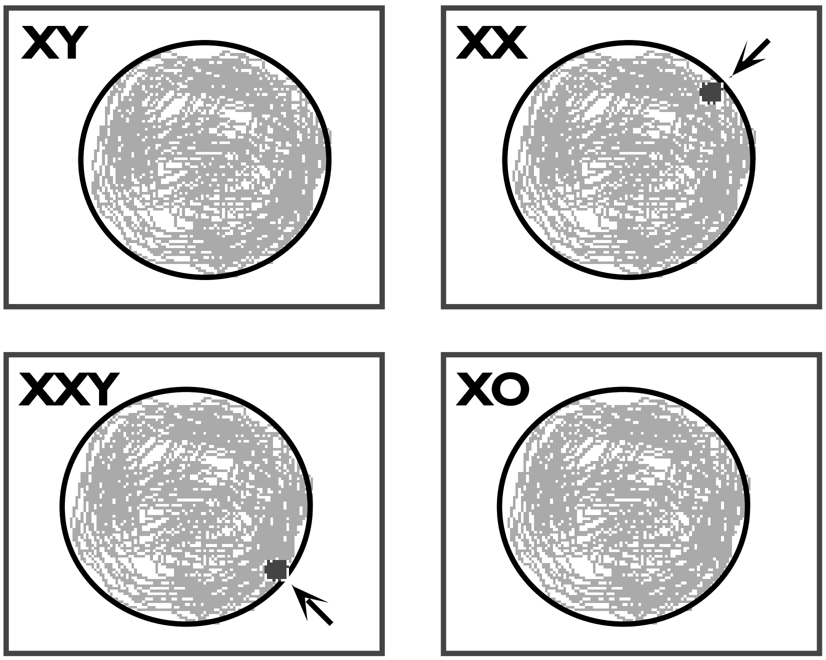
Predicted phenotype:

Actual phenotype:

The Lyon hypothesis

- 1949 – Murray Barr: "sex chromatin" in cells from female mammals

- 1959 – sex chromatin **present** in XXY males, **absent** in XO females



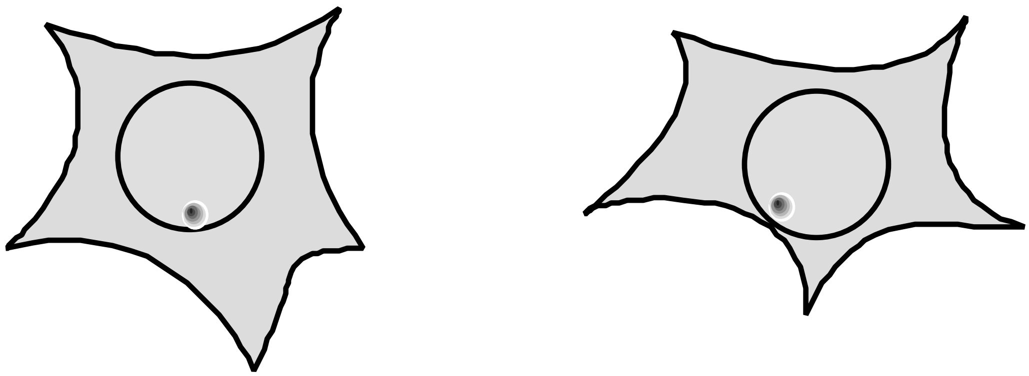
- 1961 – Mary Lyon: **inactive-X hypothesis**
  - condensed X is genetically **inactive**
  - inactivation early in development
  - inactivation **independent** and **random** in each embryonic cell

Evidence supporting the hypothesis: correlating late-replicating X with inactive allele

Fibroblast cells from female **mule**; look at expression of G6PD gene…

Which X late-replicating?

Which form of G6PD present?



Consequences of X chromosome inactivation (explaining the puzzles):

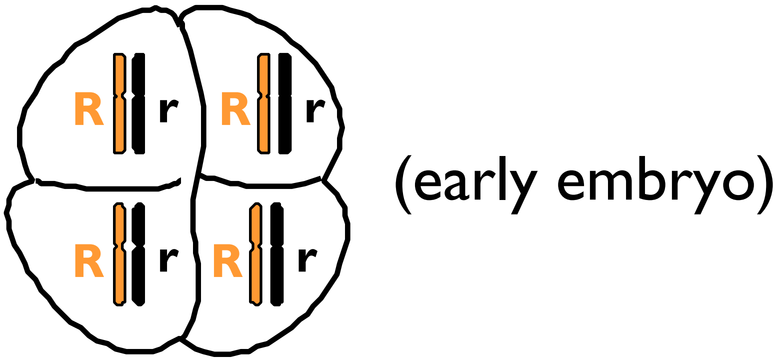
- **Dosage compensation** – Only one X chromosome genetically active

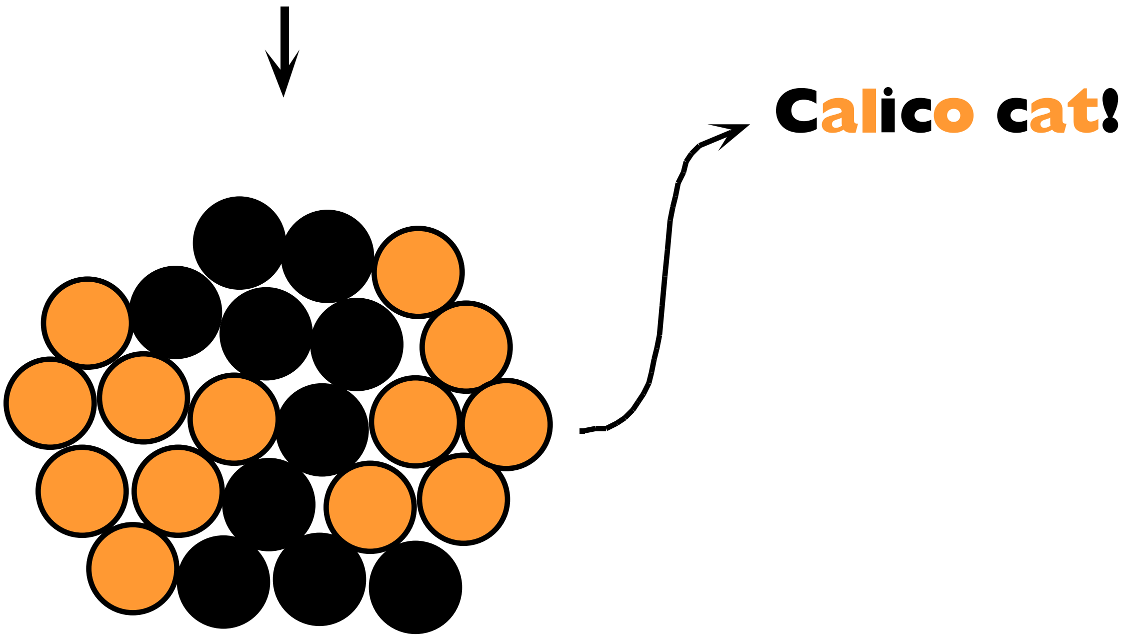
- **Mosaic** expression pattern

    - **Example 1:** the unexpected pedigree (Becker dystrophy)

◇ **Example 2:** Making a calico cat

X-linked coat color gene





Mechanism of X chromosome inactivation?

- **Selection** of one X…
- …**inactivation** of the others
- **Propagation/maintenance** of inactive state

Dosage compensation in other species

- *Drosophila*: up-regulation of X-linked genes

- *Caenorhabditis elegans*: down-regulation of X-linked genes

Mitotic recombination

Rare relative to meiotic recombination

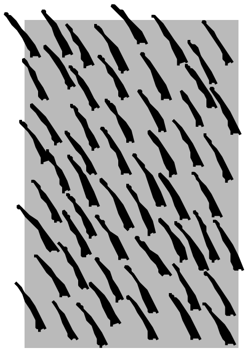
Discovery: Curt Stern, 1936

Linked genes **singed bristles** and **yellow body**

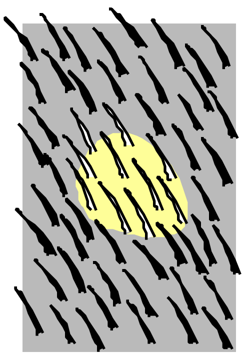
$\frac{+ \quad y}{sn \quad +}$ double heterozygote in trans configuration

> **Exercise:** Design an experiment to confirm the trans configuration

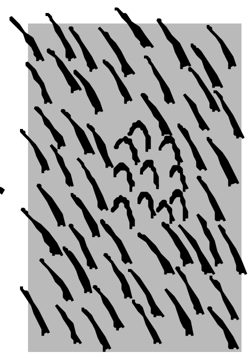
Normal



Occasionally:

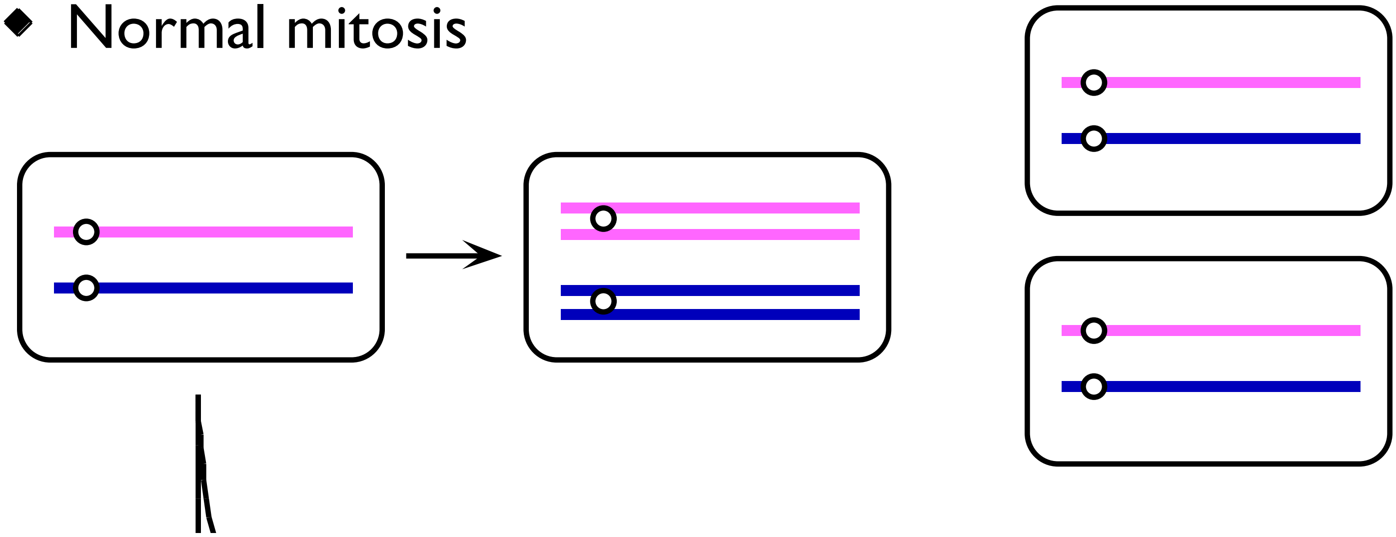


or

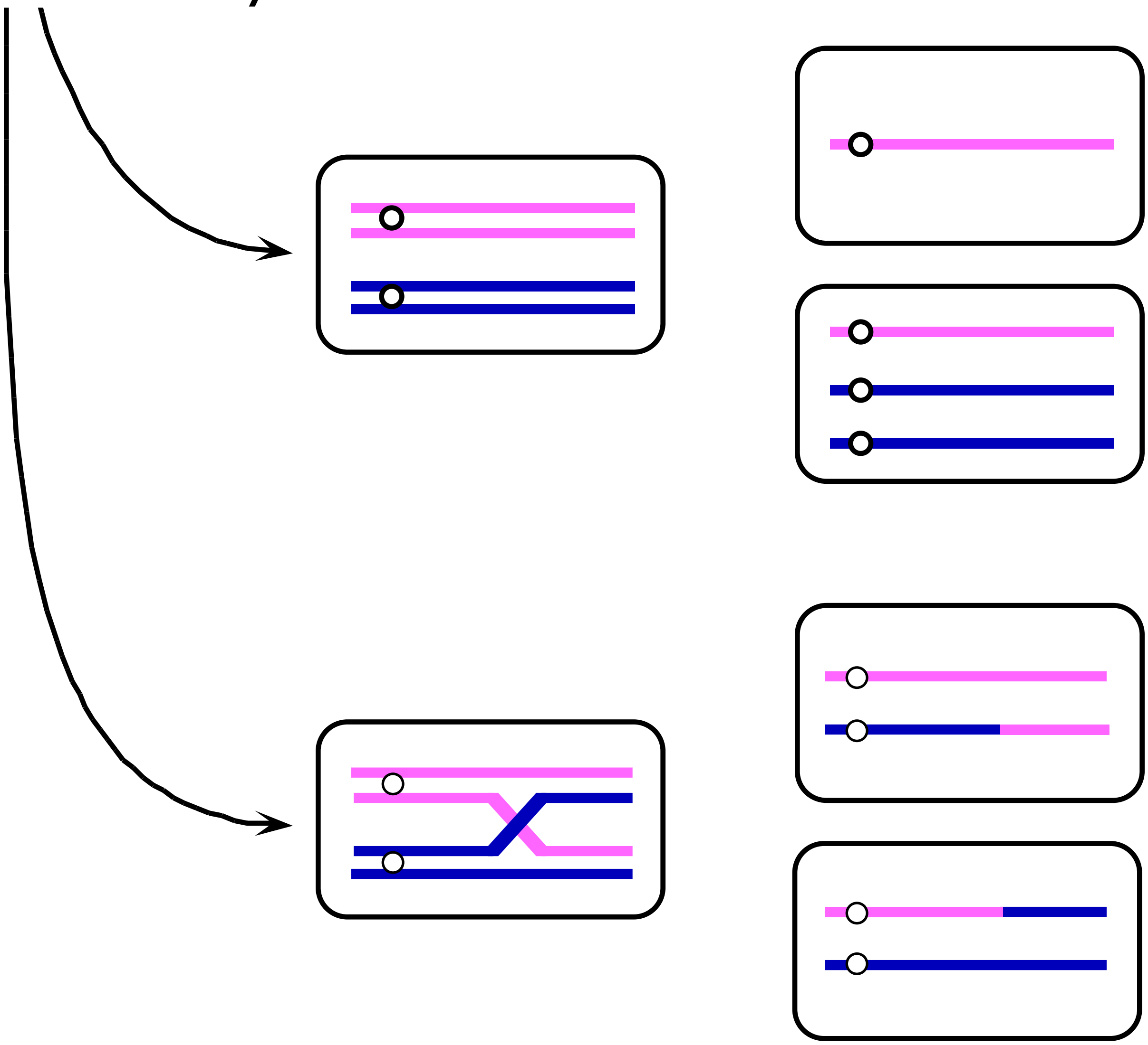


Stern's explanation

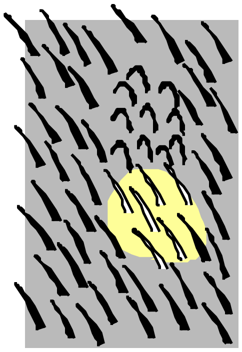
- Normal mitosis

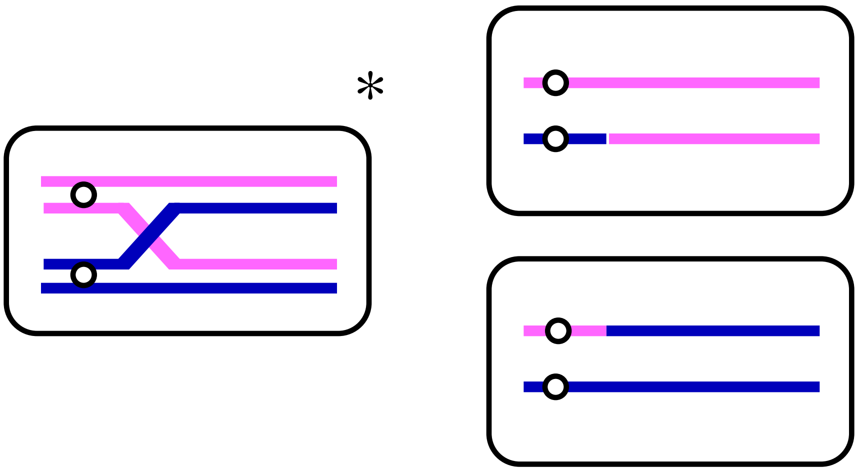


- but occasionally…



Rare twin spots:





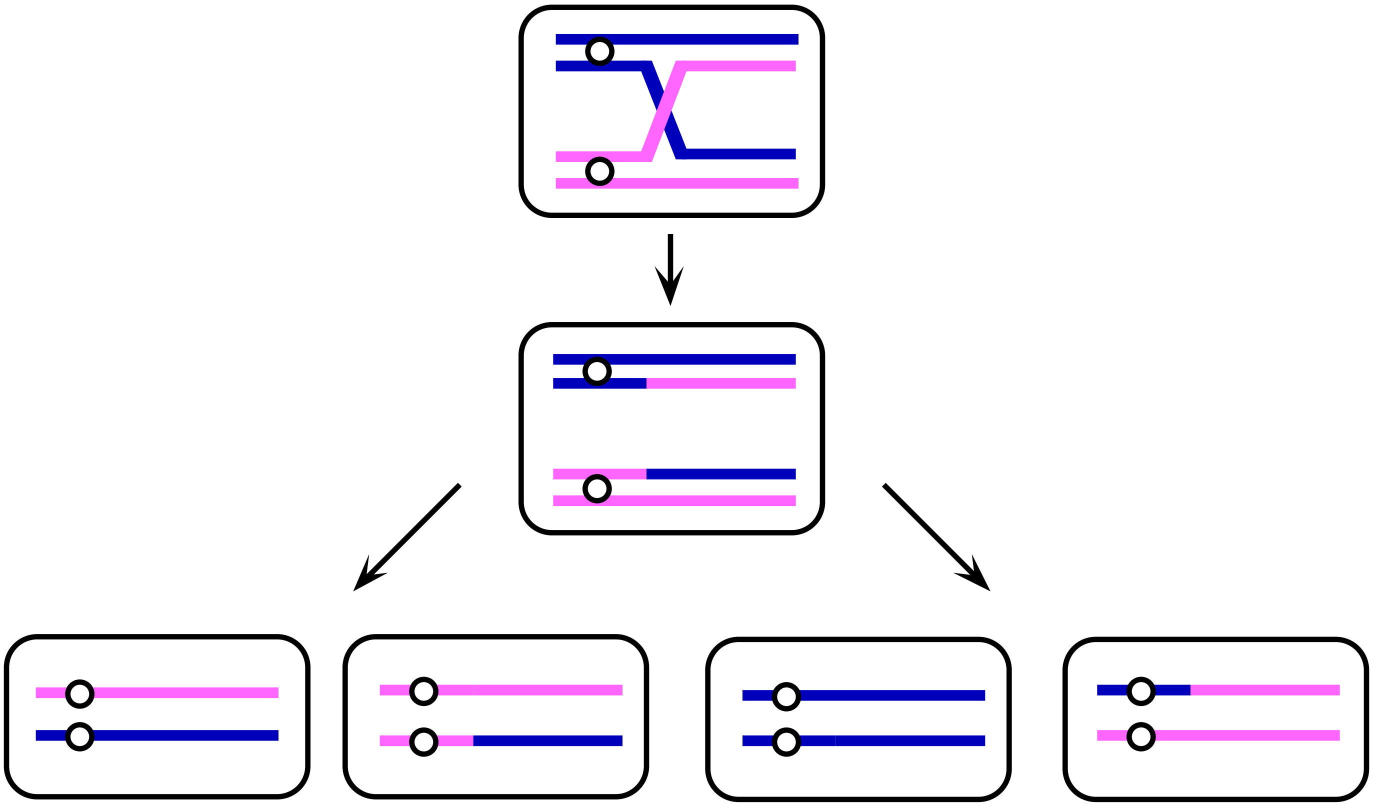
Exercise: This cell is shown to be undergoing mitotic recombination after completion of S phase (how can we tell from the diagram)? How can you tell from the products of the division that the recombination did indeed occur post-S phase?

Significance for human health?

Suppose we're talking about a recessive disease allele…

"Loss of heterozygosity"

e.g., Retinoblastoma, Wilms tumor



Sporadic cases—

Inherited form—

Explanation?

"2-hit kinetics"

$Rb^+/Rb^+ \longrightarrow Rb^+/rb \longrightarrow rb/rb$

"1-hit kinetics"

$Rb^+/rb \longrightarrow rb/rb$

Applications

- Mapping – requency of "spots" proportional to map distance

  - Mapping *centromeres* – can you get twin spots?



Caution: These are *mitotic recombination* frequencies!

- Studying development, recessive lethal alleles

- Assay for genotoxic agents – “SMART”

Properties

- Proliferation

- Metastasis

Demonstration of the genetic basis of cancer...

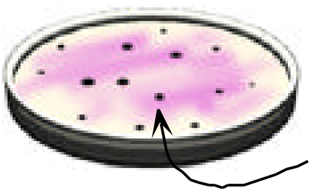
Can DNA from cancer cells transform normal cells to cancer cells?

The experiment:



Normal tissue culture cells:
monolayer

↓ *human bladder cancer DNA*



Cell **foci** –
Loss of contact inhibition!

↓

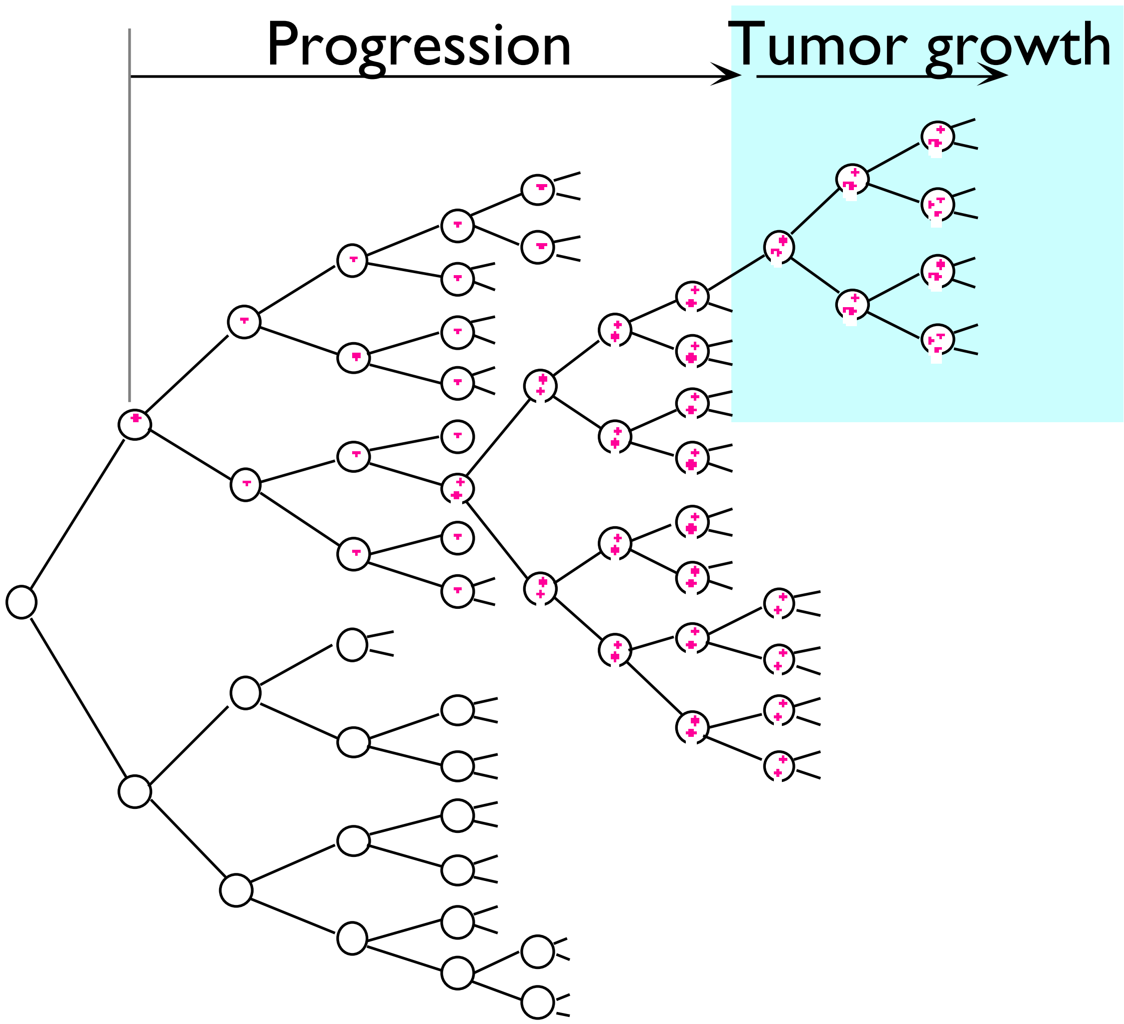
Compare transformed cell DNA with normal cell DNA

↓

single base change (G ➡ T): glycine ➡ valine

Interpreting the experiment: Only a single change to cause cancer??

Multiple mutations needed…



But what about the retinoblastoma example?

Inheritance of oncogene – predisposition to cancer, not inheritance of cancer

What does predisposition mean?

Suppose a particular form of cancer requires 4 mutations…

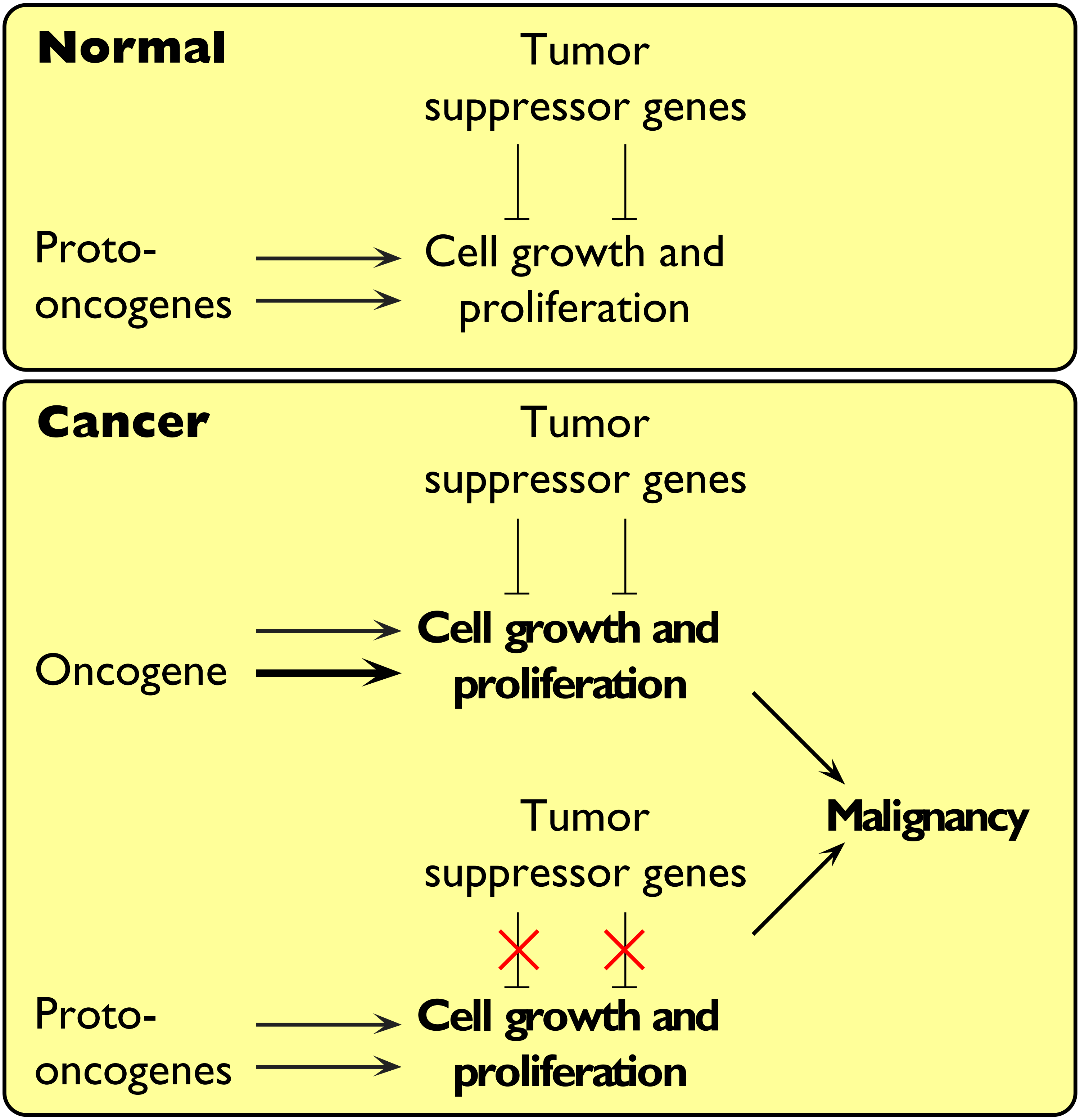
- Mutation rate ≈ 10^{-5}/cell generation
- Probability of all 4 mutations ≈

- Cell divisions to make adult human ≈ 10^{14}
- Probability of getting cancer ≈

- If one mutation has already occurred (inherited):

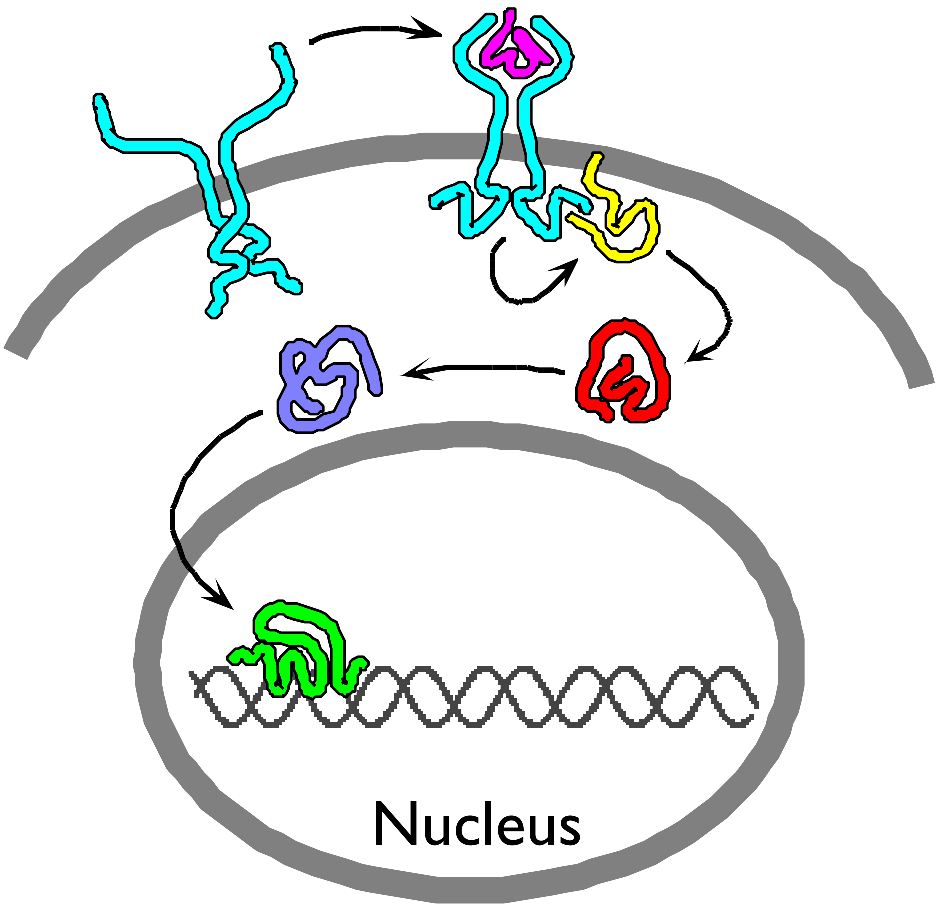
Cancer – from mutations in:

- proto oncogenes
- tumor suppressor genes
- DNA repair/maintenance genes



Proto oncogenes

- Genes that promote cell proliferation
- Often involved with signal transduction and transcription activation

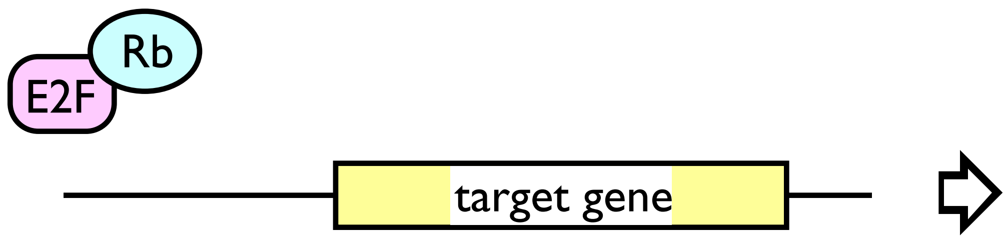


Inappropriate activation – gain of function

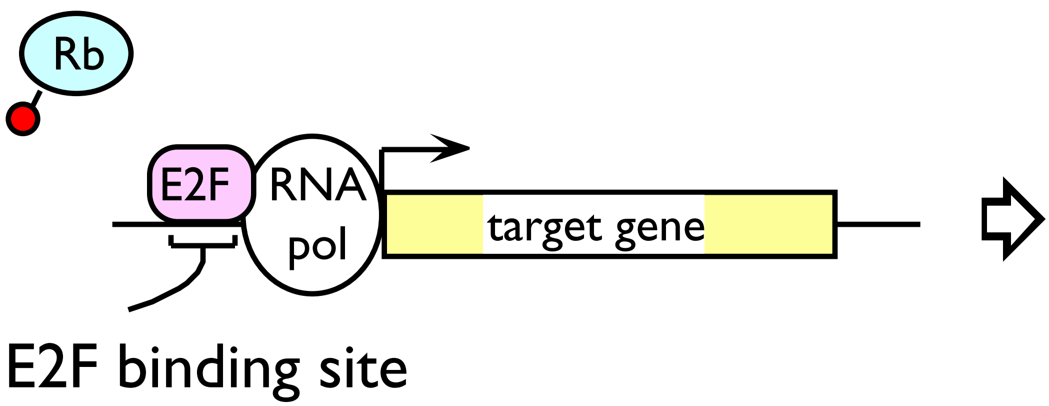
Tumor suppressors – regulate cell proliferation

e.g., E2F transcription factor: promotes G1 ⇨ S phase transition

Hypothesis: Rb protein forms complex with E2F, preventing transcription…



…but **phosphorylated** Rb protein cannot bind to E2F protein



inactivation – recessive loss-of-function

Checkpoint defects and cancer

- **p53 and response to DNA damage:**

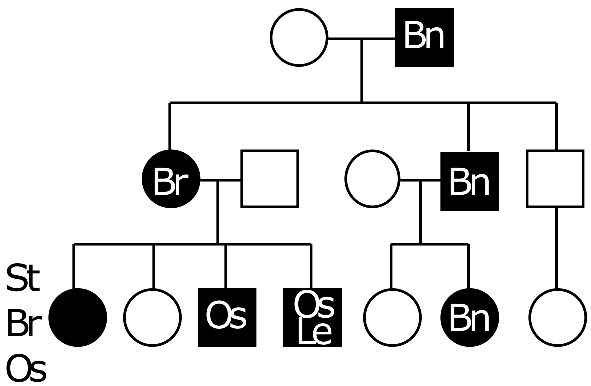
    - p53 synthesis ↑ (translational control)

    - cell cycle blocked

    - sometimes: apoptosis (programmed cell death)

Checkpoint defects may be associated with multiple forms of cancer

e.g., **Li-Fraumeni syndrome** – p53



DNA repair defects and cancer

Discovery of mismatch repair defects in human cancer…

Richard Kolodner, 1992-93

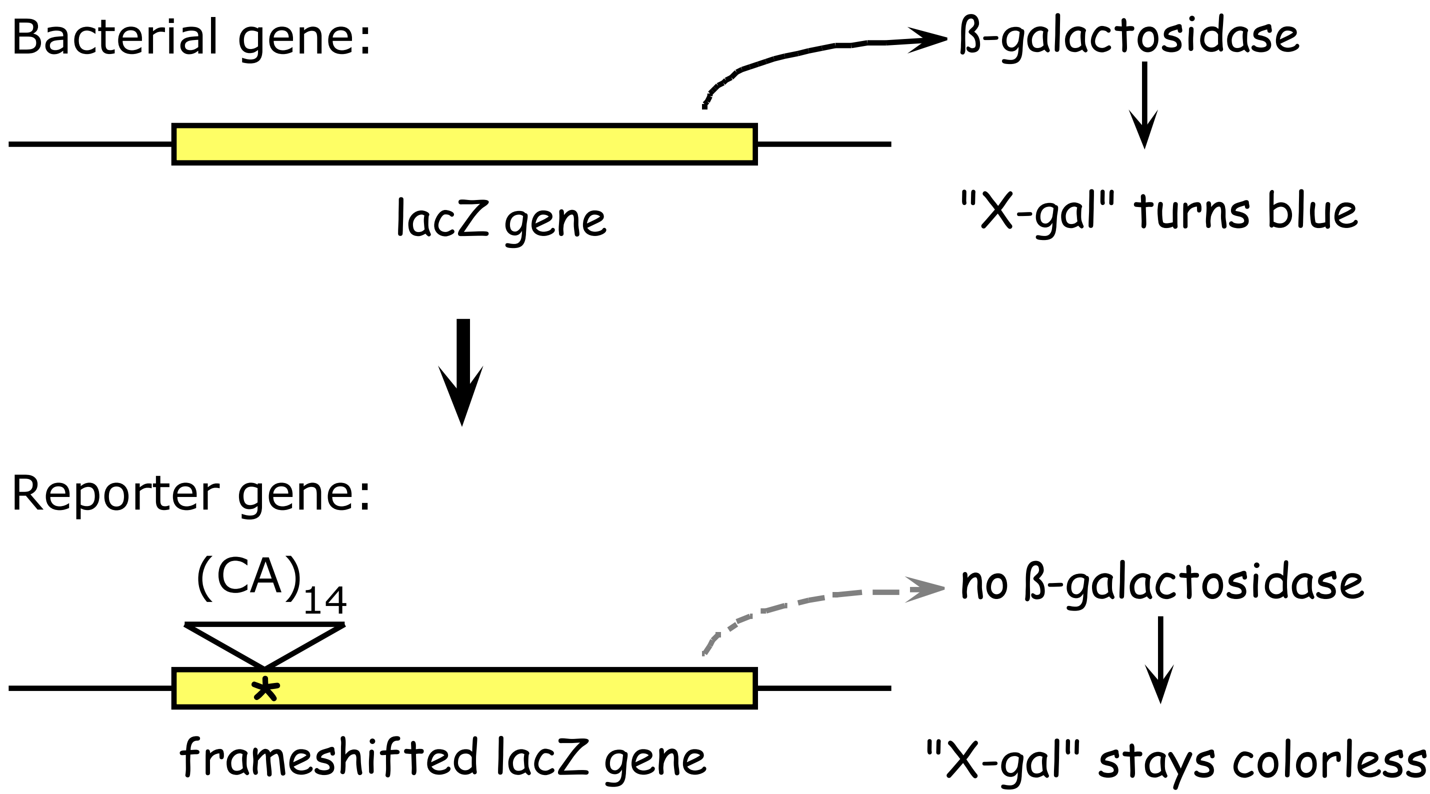
Yeast mismatch repair genes similar to E. coli’s?

Related gene in humans – Associated with HNPCC (hereditary nonpolyposis colon cancer)

Bert Vogelstein, 1993: Increase in replication errors in HNPCC cells?

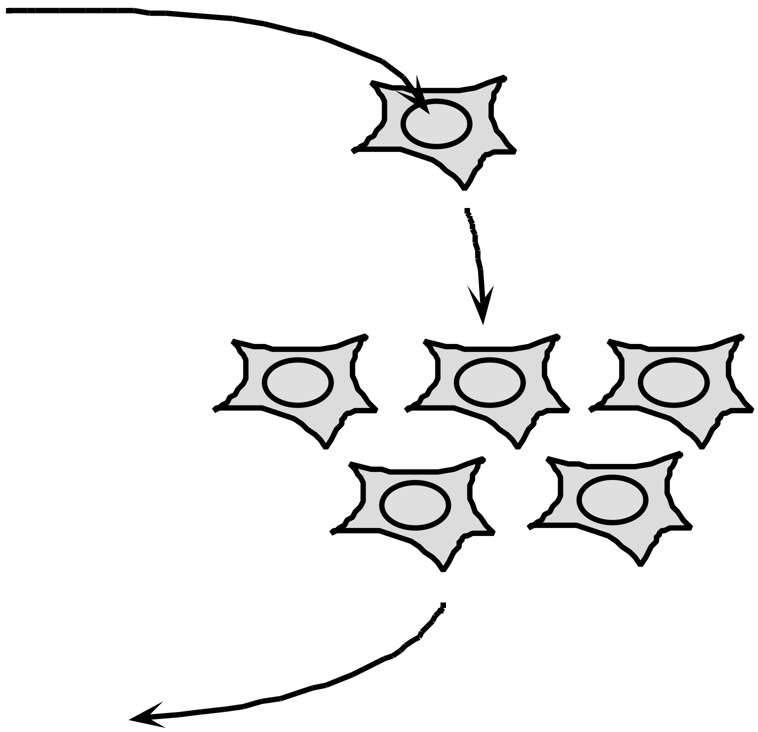
Strategy: Engineer a **reporter gene** that could cause a colorless substrate to become colored… but only if a specific kind of mutation has occurred

Engineering the reporter gene



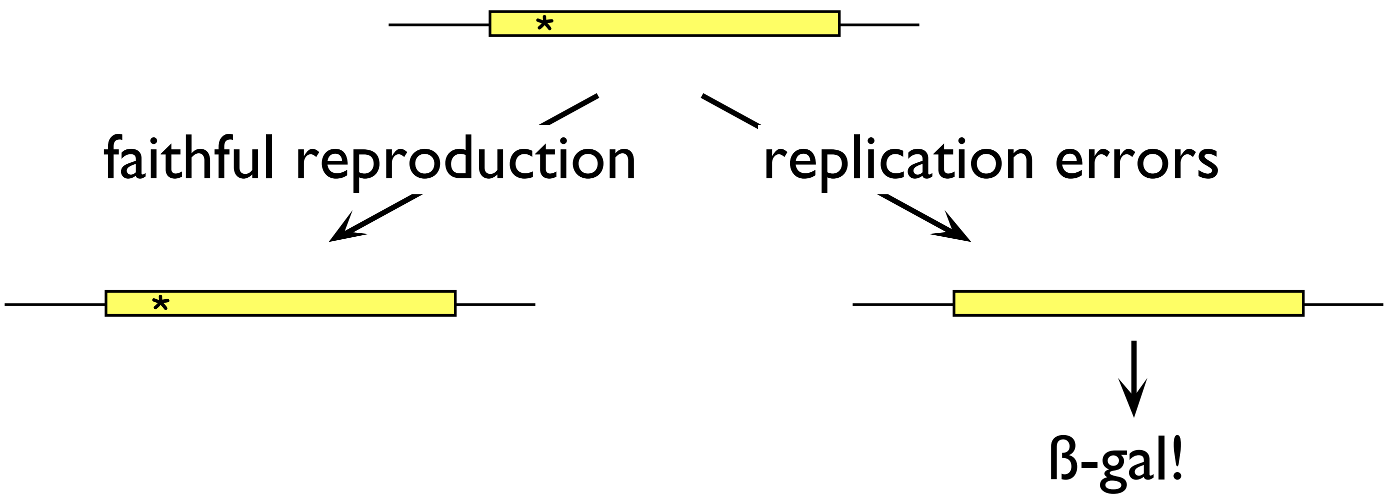
The experiment

Reporter gene



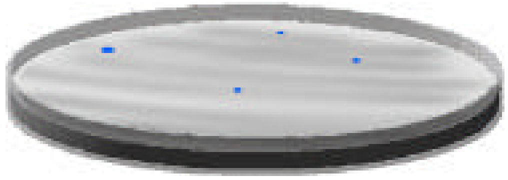
Transfer to E. coli:
Blue colonies?

The prediction

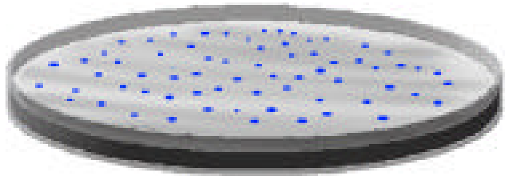


The result

Normal cells →



HNPCC cells →



Replication error rate ~100x up in tumor cells!

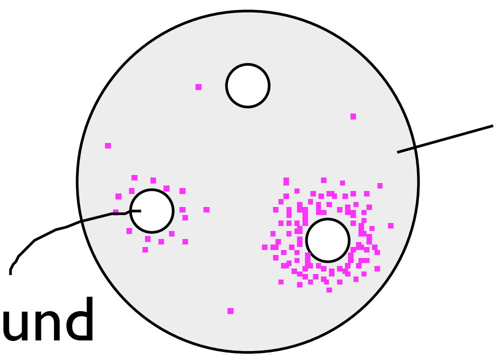
Testing for mutagens (...potential carcinogens)

The Ames test …Bruce Ames

Premise: Start with **his$^-$** Salmonella mutants (no growth w/o histidine)

base substitution
frameshift

treat with test compound:
his$^+$ revertants?



Cancer drug screening: The
"**Seattle Project**"

Lee Hartwell & Stephen Friend

Premise: Use yeast mutants to screen chemotherapeutic agents for specific defects

Practice questions

1. A tumor the size of a marble, about 1 cubic centimeter in volume, may contain 10^9 cells. How many cell *generations* (starting from a single cell) are required to produce this tumor? How many cell *divisions* were involved?

2. Some uterine tumors consist of as many as 10^{11} cells. In women heterozygous for a particular X-linked gene, researchers have discovered that *every* cell of such a tumor has the *same* active X-linked allele. Explain this observation in terms of the Lyon hypothesis.

3. Although it is generally agreed that the path to malignancy is a multistep process, Weinberg and his colleagues were able to transform tissue culture cells in *one* step. Suggest an explanation for this apparent discrepancy.

4. The proto-oncogene erbB encodes the cell surface receptor for a growth factor. Binding of growth factor to the receptor signals the cell to divide. Speculate on how a mutation in the erbB proto-oncogene might lead to malignancy.

5. Researchers have found that breast cancer is not common among *homozygotes* affected with ataxia-telangiectasia, but breast cancer is the most frequent type of cancer among *heterozygotes* for A-T. The researchers think that this oddity might be a consequence of the ages of the people in the two groups. Can you give a reasonable explanation?

Discovery of **cytoplasmic inheritance**

Boris Ephrussi, ~1949: Genetics of respiration in yeast

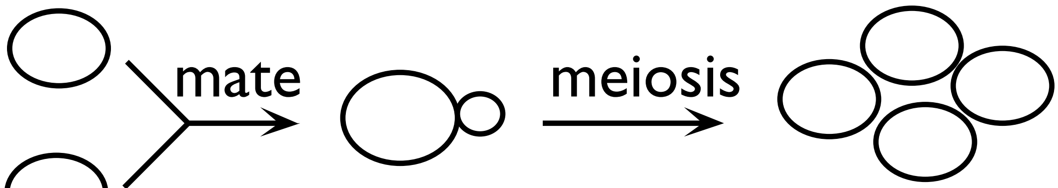
- Respiration: oxidative breakdown of nutrients to release energy; coupled to ATP synthesis to allow cells to use the released energy

- Site of oxidative phosphorylation:

- "**Petite**" and "**grande**" yeast

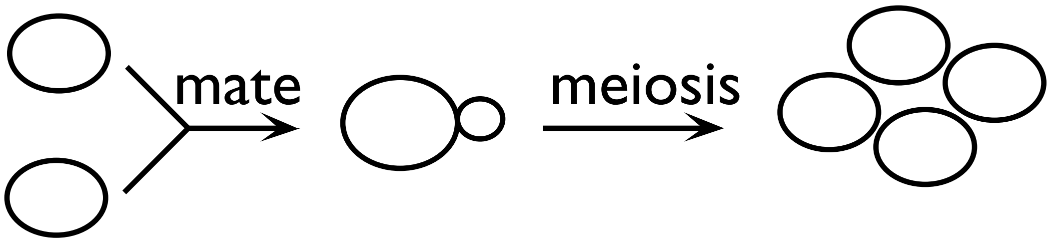
Two kinds of “petite” mutations:

- Normal **Mendelian** inheritance



Predicted genotype:

- **Non-Mendelian** inheritance



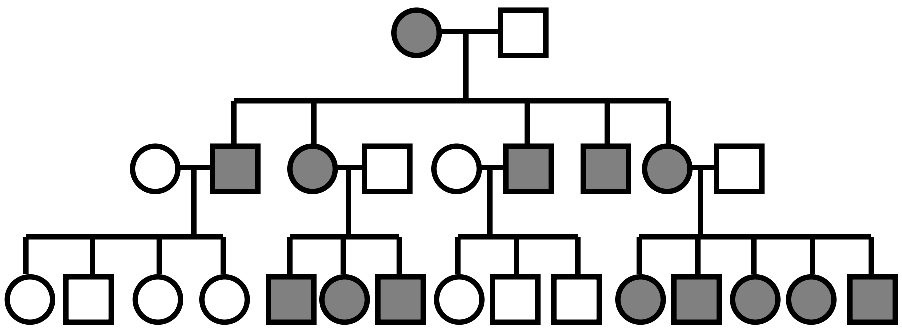
Ephrussi’s explanation: cytoplasmic inheritance; predicted “rho factor” in mitochondria

The mitochondrial genome

- Yeast

- Human
  - 37 genes
  - Expression coordinated with nuclear genes

Maternal inheritance of mtDNA



Explanation: Mitochondrial contribution of sperm vs. egg

Mitochondrial DNA disorders in humans

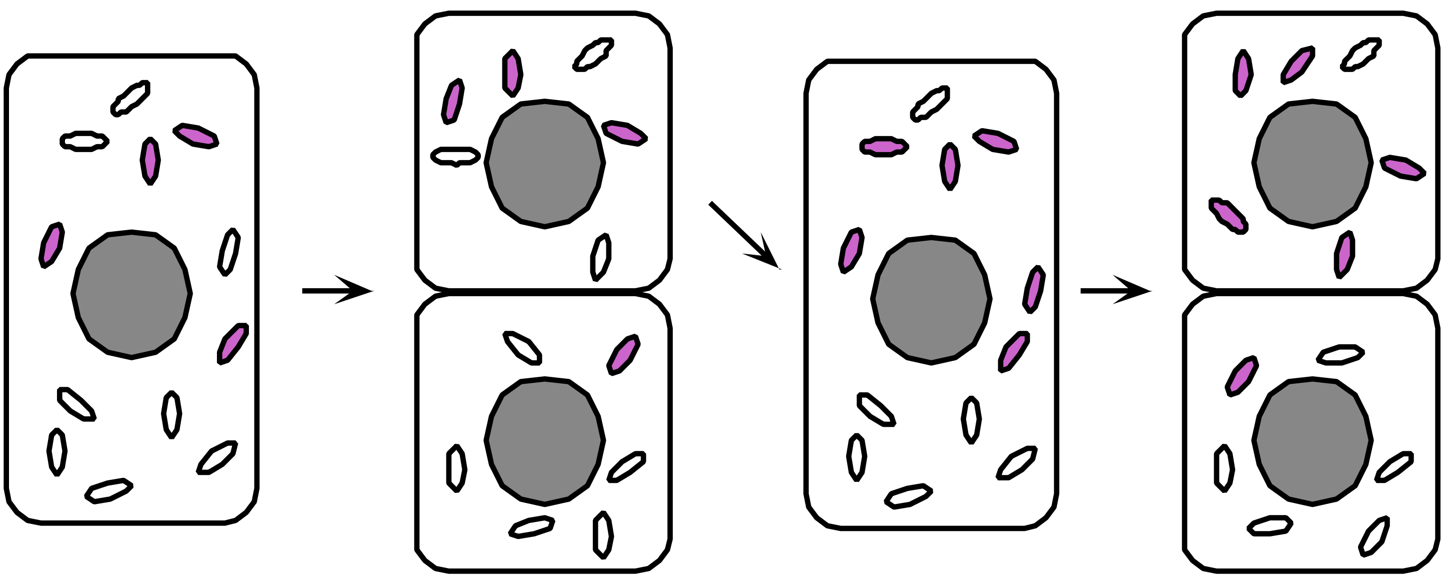
- inherited
- spontaneous mutations in egg or early embryo
- somatic mutations during the life of the individual

But with >>100's of mtDNAs per cell, how could sporadic (recessive) changes give a disease phenotype?

- Cumulative changes –

- Impaired central function (e.g., protein synthesis)

- Random segregation of mitochondria: **homoplasmy** from **heteroplasmy**

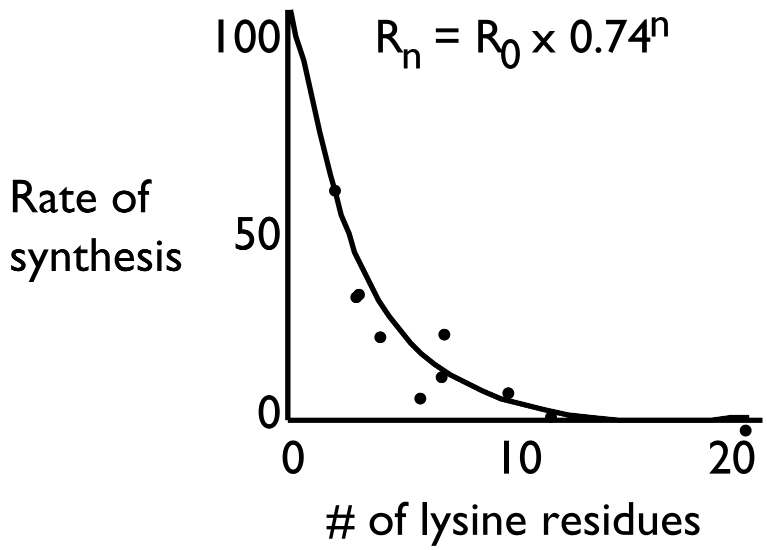


MERRF (Myoclonic epilepsy and ragged red fibers):

Defect:

non-functional lysine tRNA ($tRNA^{Lys}$)

Different proteins affected to different extents:



Interaction with the environment

- Nonsyndromic deafness

- Mutation: A1555G — in 12S rRNA gene

- Variable age-of-onset, severity

- Common thread? Correlation between manifestation of disorder and treatment with aminoglycosides

Why the high mutation rate?

- little or no DNA repair, poor error-correction
- proximity of oxidative phosphorylation centers – free radicals!
- A connection with aging?

Practical applications

- Forensics
- Tracing population migrations

Modified dihybrid ratios

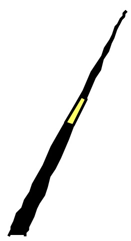
– a single character determined by the action of two genes

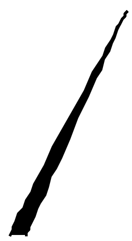
- **Epistasis**
  - ◇ Recessive epistasis – e.g., mouse coat color (see lecture 2)

Aa x Aa

↓

3 agouti: 1 black





A second gene influencing coat color: C

CC x cc

↓

Cc

↓

3 C : 1 c

Now both genes together:

AaCc x AaCc

↓

A_C_ A_cc aaC_ aacc

Siamese cats…

- **Dominant epistasis** – e.g., squash color

White x Green

↓

White

↓

12 White : 3 Yellow : 1 Green

- **Complementation**

e.g., flower color in sweet pea

Variety 1 x Variety 2

white flowers white flowers

↓

Purple flowers

↓

9 purple : 7 white

- **Suppression**

  e.g., eye color in *Drosophila*

pd pd Su Su x Pd Pd su su

purple red

↓

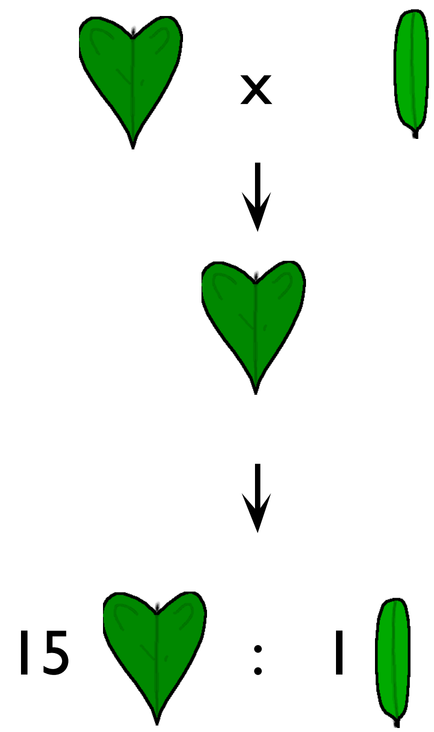
Pd pd Su su red

↓

13 red : 3 purple

- **Redundant genes**

  Shepherd's purse:
  Heart-shaped or narrow fruit



- Distinguishing between **modified dihybrid** and **monohybrid**

- Distinguishing between **modified dihybrid** and **linkage**

Genetic analysis - I

The goal: understanding a biological process

The approach: break the system one component at a time; ask how it's broken (phenotype)

The tools

- Mutations
- Recombination

“Breaking” the system – mutagenesis of a large population

- few (usually, ≤ 1) mutations per individual
- for each gene, at least a few individuals (in the population) who have a mutation in that gene

Mutagenesis:

Screen vs. **selection** – identifying the mutants you are interested in

- Screen –

- Selection –

Examples

- The interview – finding a translator
  - Screen
  - Selection

- Fly, fly away – wingless fly mutants
  - Screen
  - Selection

Bacterial transformation to antibiotic resistance – selection or screen?

Vogelstein's assay for replication errors – selection or screen?

Determining the number of genes involved in a process…

- **Map** each mutation

- **Complementation** test
  - Do Mutant 1 and Mutant 2 have mutations in the same gene or in different genes?

  Example 1 – feather coloring in peacock… suppose you've identified two recessive mutations that cause loss of color (white chickens). Are the mutations in the same gene or in separate genes?

Example 2 – *Drosophila* eye color

To find which mutations are in the same gene vs. different genes…

Make all possible heterozygotes, check phenotypes of females

	white	prune	apricot	buff	cherry	eosin	ruby
white	-	+	-	-	-	-	+
prune	+	-	+	+	+	+	+
apricot	-	+	-	-	-	-	+
buff	-	+	-	-	-	-	+
cherry	-	+	-	-	-	-	+
eosin	-	+	-	-	-	-	+
ruby	+	+	+	+	+	+	-

+ = wildtype, - = mutant

Interpreting the results: **complementation groups** –

Group together those mutations that **fail** to complement **other** mutations

Cautionary notes:

- lethals
- dominant mutations

Determining the order of action of genes

- One approach: provide the intermediate that the mutant can't make…

  [Analogy: restoring an assembly line]

- Disadvantage: need to know the intermediates in the pathway

  Example: arginine synthesis defects in Neurospora

arg-1, *arg-2*, *arg-3*: wildtype alleles of all 3 needed for Arg synthesis

6 possible linear pathways:

Precursor —*arg-1*→ inter-mediate 1 —*arg-2*→ inter-mediate 2 —*arg-3*→ Arginine

Precursor —*arg-1*→ inter-mediate 1 —*arg-3*→ inter-mediate 2 —*arg-2*→ Arginine

etc.

Predictions:

If the first pathway is correct,

◇ arg-1 mutants –

◇ arg-2, arg-3 mutants –

Intermediates: ornithine, citrulline

Experiment:

Add one supplement at a time to the growth medium; ask: does the mutant show growth? ("+" = growth, "-" = no growth)

	Supplement:			
	None	Ornithine	Citrulline	Arginine
Wildtype	+	+	+	+
arg-1	-	+	+	+
arg-2	-	-	+	+
arg-3	-	-	-	+

Interpretation:

arg-3 is not rescued by of the intermediates—

arg-2 is helped by citrulline but not by ornithine—

arg-1 can grow on any of the intermediates—

The correct pathway:

Precursor → Ornithine → Citrulline → Arginine

A genetic way of ordering the pathway:
Epistasis analysis
Compare double mutant phenotype with single mutants

Advantage: don't need to know intermediates, just need distinct phenotypes for the various mutations

e.g., coat color in mammals

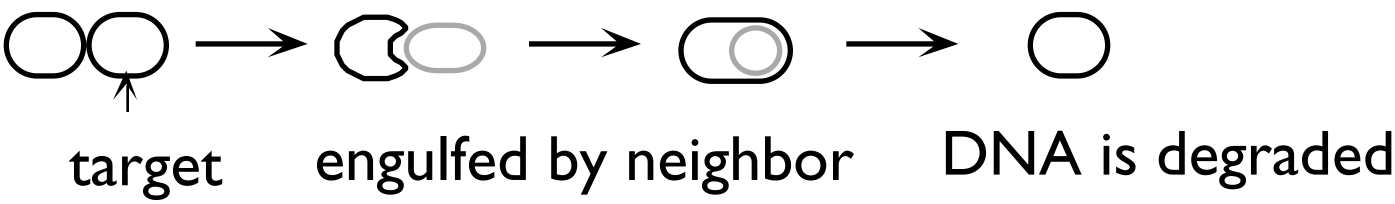
Consider two genes: **C** and **E**

ccE_ : albino (no pigment)
C_ee : no color in coat

cc ee double mutant: albino

Interpretation:

Another example: programmed cell death (apoptosis) in *C. elegans*



Mutant gene	**phenotype**
ced-3	cells live
ced-2	cells die, not engulfed
nuc-1	cells die and engulfed, DNA not degraded
Double mutants	
ced-3, ced-2	cells live
ced-2, nuc-1	cells die, but are not engulfed
ced-3, nuc-1	cells live

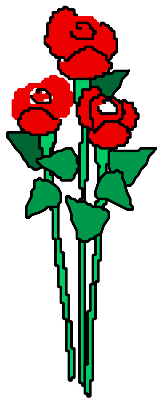
An example of a **negative interaction**:
Rb and E2F

rb^- : cells enters S phase

$E2F^-$: cell does not enter S phase

double mutant: cell does not enter S phase

An exercise:



Mutational analysis of flower color was undertaken in a plant species that normally makes red flowers. The mutations fell in three complementation groups: **A**, **B**, and **D**. The phenotypes of single and double null mutants are listed:

Mutant	Phenotype
$\mathbf{a^-}$	purple flowers
$\mathbf{b^-}$	red flowers
$\mathbf{d^-}$	white flowers (no color)
$\mathbf{a^-\ b^-}$	red flowers
$\mathbf{a^-\ d^-}$	white flowers
$\mathbf{b^-\ d^-}$	white flowers

Deduce the pathway of flower color production.

Extra challenge: How might the $\mathbf{b^-}$ mutant have been detected?

To be discussed on Monday, Nov. 22

Gene regulation

Genetics 371B Lecture 27 | 17 Nov. 1999

Why regulate genes?

Control points:

Two modes of control:

Positive control	**Negative control**
Gene **OFF** until activator turns it **ON**	Gene **ON** until repressor turns it **OFF**

François Jacob
Jacques Monod] **lac operon**

E. coli – can metabolize lactose (disaccharide, galactose-o-glucose)

BUT… synthesis of ß-gal is regulated —

Carbon source	**ß-gal enzyme activity/cell**
glycerol	
lactose	

⟹ Lactose is an **inducer** of ß-gal production

[An artificial inducer: isopropyl thiogalactoside, **IPTG**]

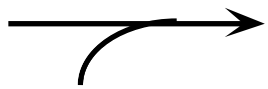
Mode of action of inducer?

- **Possibility 1:** Inducer activates already-existing ß-Gal

- **Possibility 2:** Inducer triggers fresh synthesis of ß-Gal

Experiment

Cells + lactose

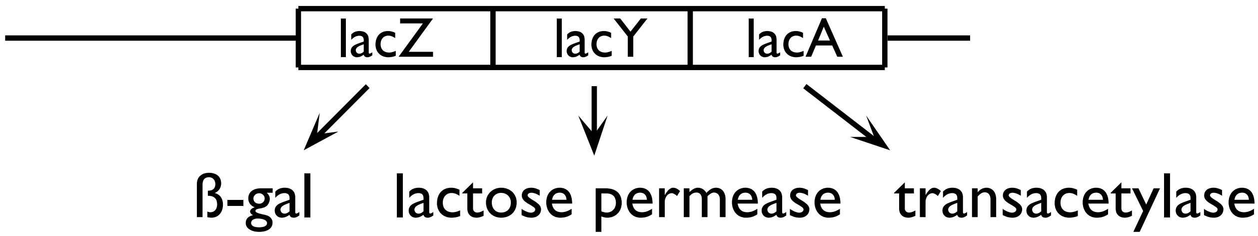


radioactive
aminoacids

Control?

From mutational analysis: three linked **structural genes**…

…coordinately regulated



Polar mutations

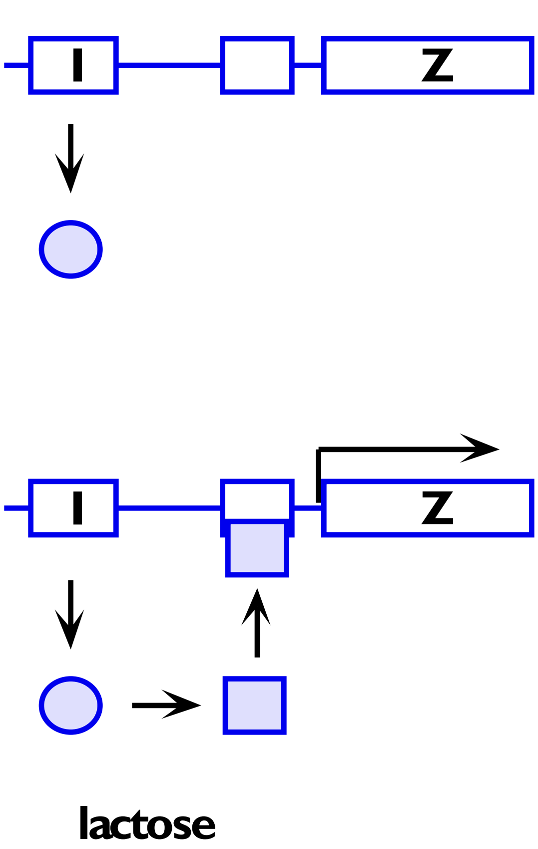
So is transcription of the lac operon under positive control or negative control? How to tell?

Some mutations: **regulation** affected

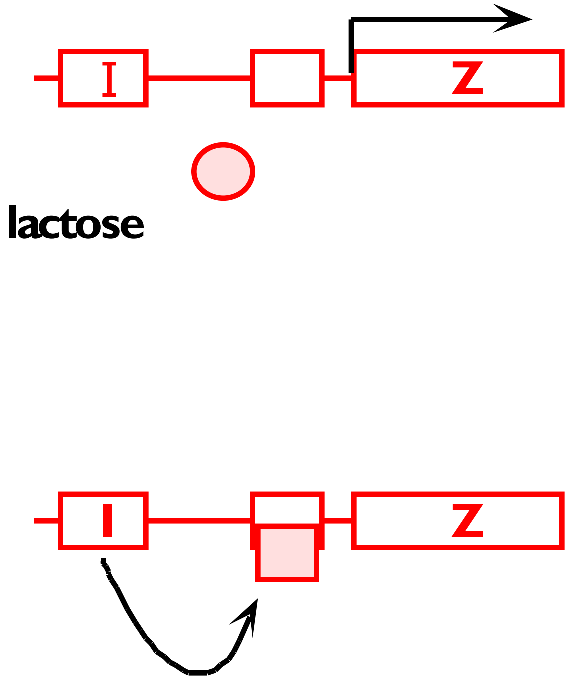
strain	ß-gal level in glycerol	ß-gal level in lactose
Wildtype		
Mutant 1		
Mutant 2		

lacI map location:

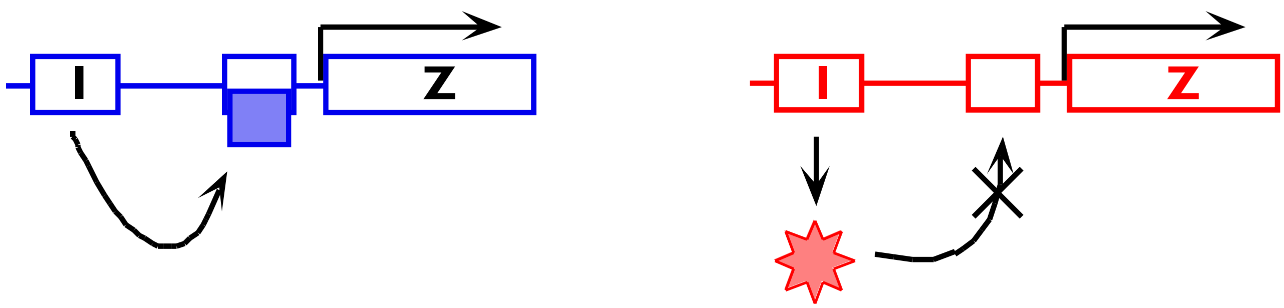
If Positive...



If Negative...

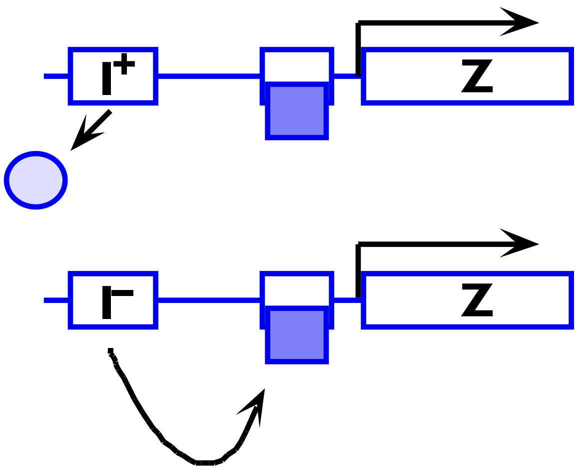


I^- constitutive mutants

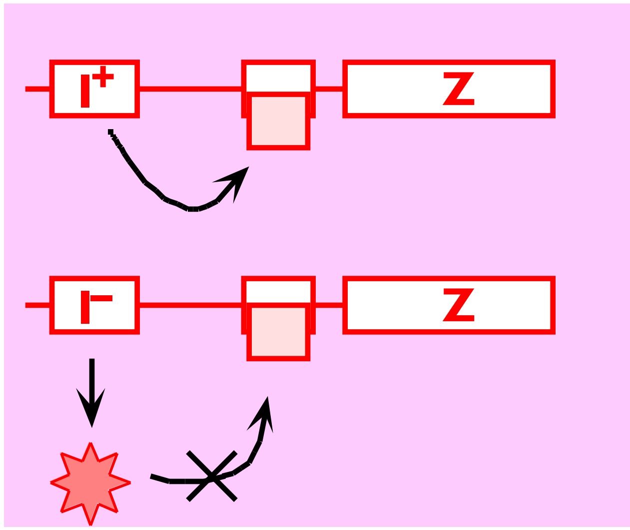


To distinguish between these two possibilities: does the I^- mutation act as a **dominant** or a **recessive** mutation?

Positive



Negative



BUT… these are bacteria

How to get “diploids” to test dominant vs recessive?

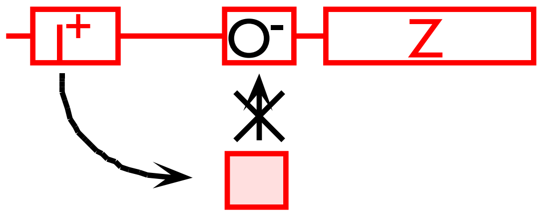
– partial diploid $\left[\begin{array}{l} I^{+}\ Z^{+} \\ I^{-}\ Z^{+} \end{array}\right.$

Implicit in the model: repressor acts in **trans**

"Super repressor" lacI^{S}:

Target of the repressor? **Operator** sequence, or lacO

Predicted phenotype of lacO mutation?



Challenge: lacO is small (24 bp) relative to lacI (1080 bp)
How to avoid getting mainly lacI^{-} mutants?

lacO acts in cis; lacO^{C} is cis-dominant

– it matters whether lacZ is "attached" to O^{+} or O^{C}

I^{+} O^{+} Z^{-} I^{+} O^{c} Z^{+}	I^{+} O^{c} Z^{-} I^{+} O^{+} Z^{+}

Gene regulation - II

Genetics 371B Lecture 28 — 17 Nov. 1999

Last time…

Negative control of transcription in the lac operon

BUT… That was in cells grown in glycerol

What if cells are grown in glucose?

Carbon source	ß-gal activity/cell
glycerol	
glycerol + lactose	
glucose	
glucose + lactose	

Glucose overrides the lacI system:

	- glucose + lactose	+ glucose + lactose
$lacI^-$		
$lacO^c$		

Why?

Mutational analysis of **catabolite repression**:

- ◇ cya$^-$
- ◇ cap$^-$

Complementation

- ◇ cya$^-$ cap$^+$ / cya$^+$ cap$^-$:

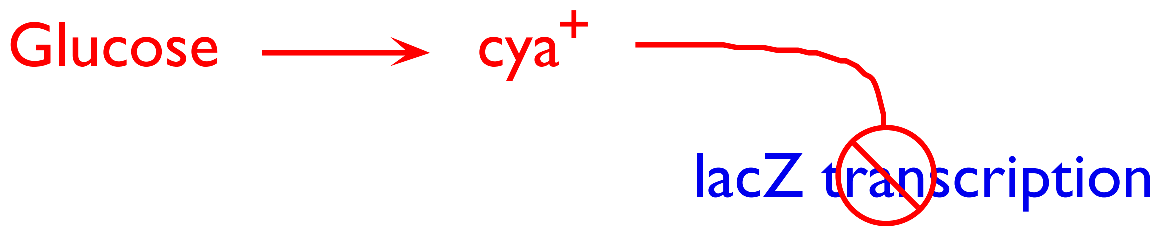
Models

- ◆ **Positive control (activation)**



cya$^-$ cells:

- ◆ **Negative control (repression)l**



cya$^-$ cells:

Test of the models:

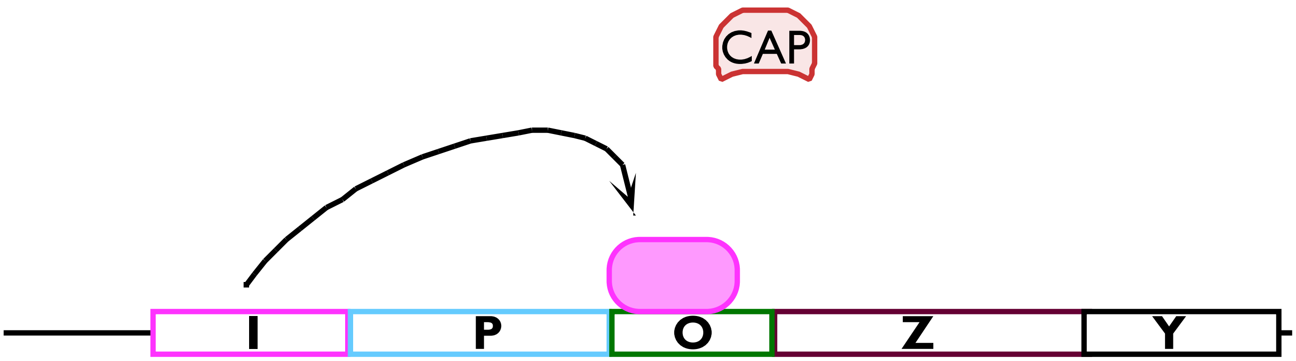
cya^- / cya^+:

What do cya and cap do?

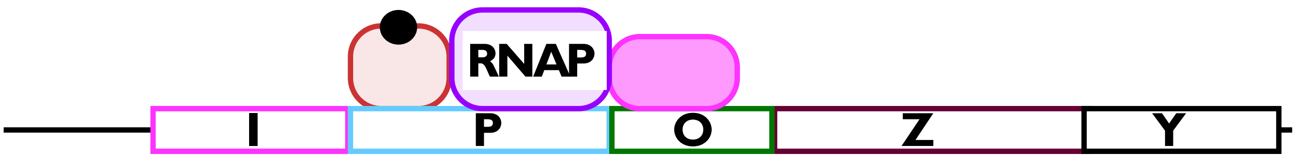
cya^+: adenylate cyclase

cap^+: catabolite activator protein (CAP)

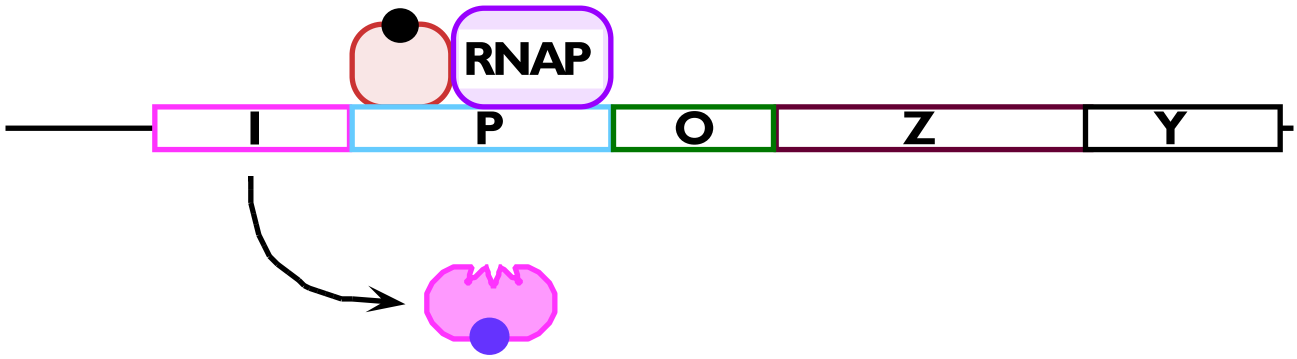
- Glucose present, lactose absent



- Glucose absent, lactose absent



- Glucose absent, lactose present



Exercise: Draw a pathway to represent regulation of the lac operon (including glucose and lactose).

Regulation of transcription in eukaryotes:

The ***GAL*** regulatory pathway in yeast

- GAL1, GAL10, GAL7 gene transcription –

- Regulatory mutations:

Strain	**Phenotype**
$gal4^-$	non-inducible
$GAL4/gal4^-$	inducible
$GAL4^c$	constitutive
$GAL4/GAL4^c$	constitutive
$gal80^c$	constitutive
$GAL80/gal80^c$	inducible
$gal4^-$ $gal80^c$	non-inducible

Interpreting...

- Is GAL4 a positive activator or a negative regulator of GAL gene transcription?

- Is GAL80 a positive activator or a negative regulator of GAL gene transcription?

- What kind of interaction do GAL4 and GAL80 have?

Developmental genetics - I

The problem faced by embryos

- **Cell fate –** determination and differentiation

Two solutions to the problem

How to distinguish between these possibilities?

Generating positional information

- Intracellular gradients

- Cell-cell signaling

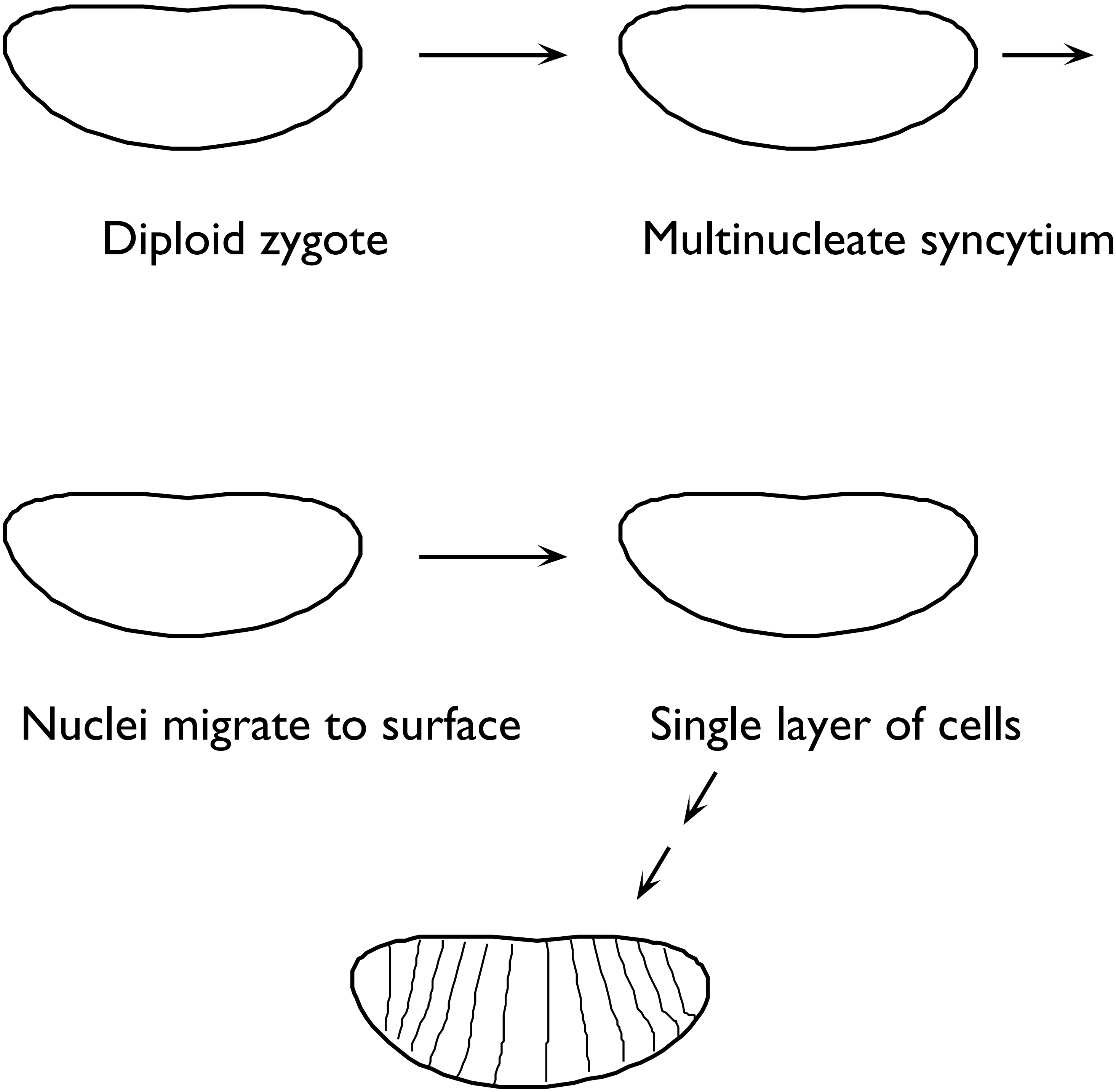
Drosophila – A model system to study development

Why Drosophila?

- large larva
- rapid development
- molecular biology and genetics

Christiane Nusslein-Volhard
Eric Wieschaus

The early Drosophila embryo:



Types of mutants identified:

- **Maternal-effect genes –** zygote phenotype determined by maternal genotype
  - e.g., bicoid, nanos, oskar
  - **Interpretation:**
- **Zygotic genes –** zygote phenotype determined by zygote genotype
  - **Interpretation:**

Zygotic gene classes:

- **Gap genes** (!) e.g., hunchback, knirps

- **Pair-rule genes** e.g., fushi-tarazu, even-skipped

- **Segment polarity genes** e.g., engrailed, hedgehog

- **Selector (segment identity) genes** e.g., Antennapedia

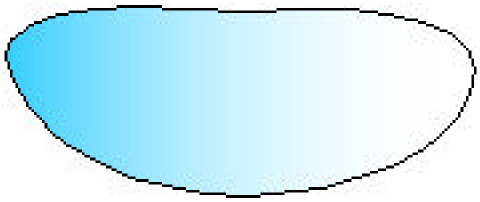
Overall strategy of body-plan formation:

- Establish polarity

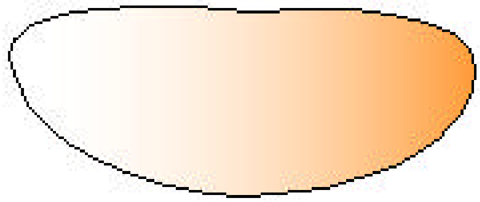
- Then: combinatorial gene expression

Step 1. Establish asymmetry (anterior-posterior, dorsal-ventral)

bicoid mRNA –

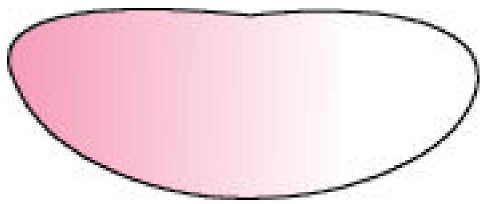


nanos mRNA –



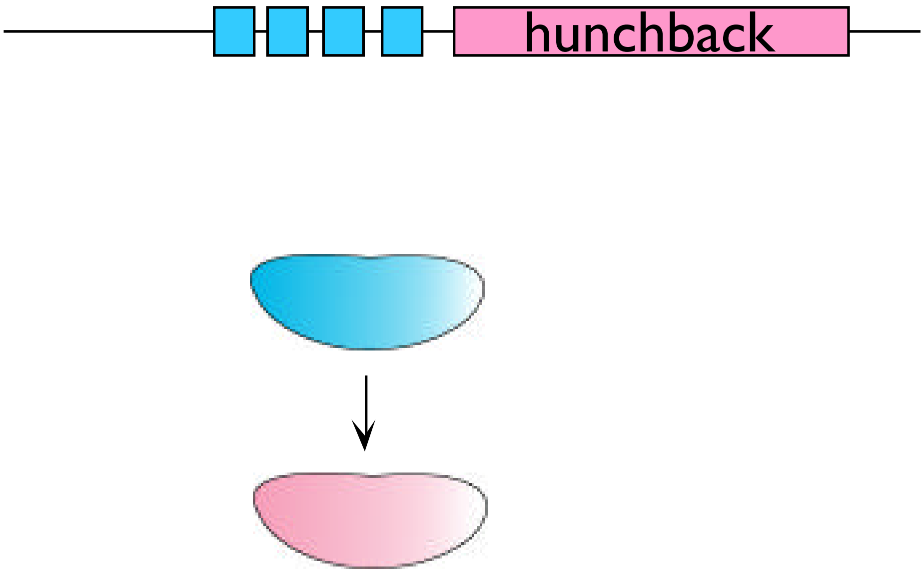
Step 2. Read positional information, make broad divisions

bicoid → *hunchback* transcription

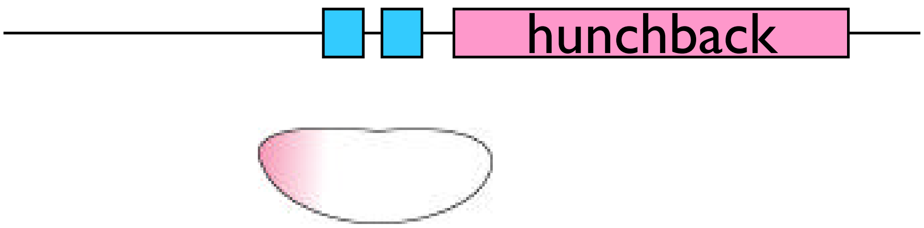


hunchback transcription: dependent on bicoid protein level

- **Expt. 1:** Overexpress bicoid



- **Expt. 2:** Reduce # of bicoid binding sites

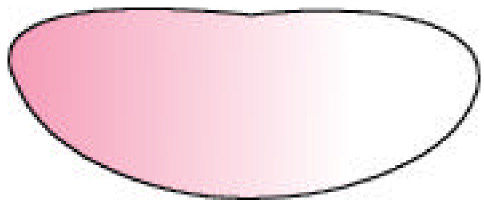


- **Expt. 3:** Inject bicoid mRNA into posterior end… your prediction?

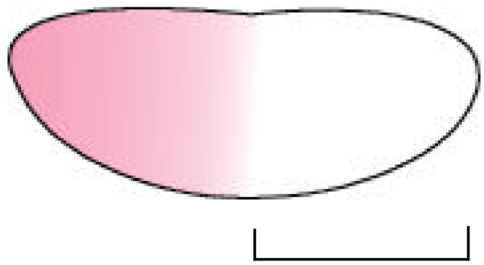
Developmental genetics - II

Genetics 371B Lecture 30 — 24 Nov. 1999

hunchback transcript:



hunchback protein:



why no hunchback protein here?

Step 3. Establish segment boundaries

gap gene mutations:

pair-rule gene mutations:

How does combinatorial expression work?

Step 4. Establish segment structure

segment-polarity gene mutations:

Step 5. Establish segment identity: selector genes

homeotic mutations:

loss-of-function mutations:

gain-of-function mutations:

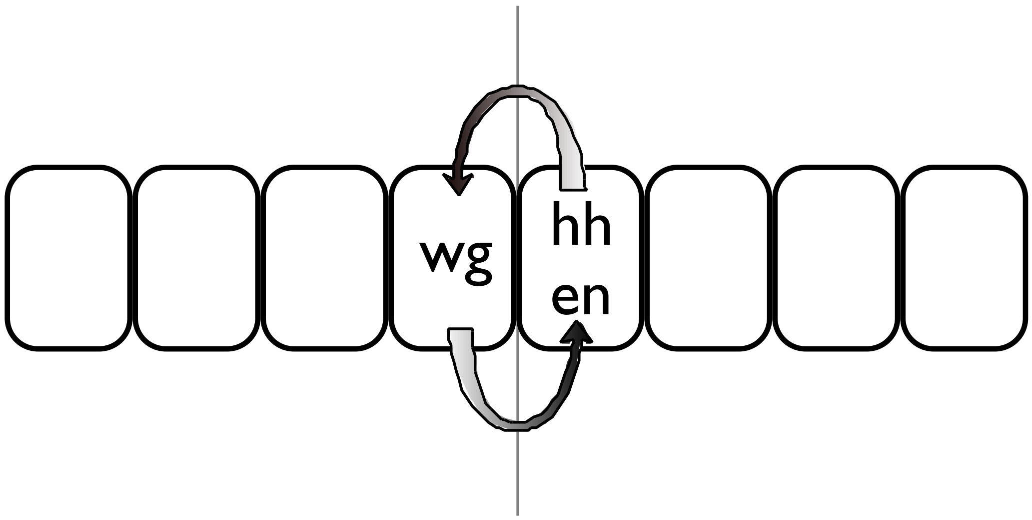
Conclusion from this mutational analysis:

The homeobox:

Remembering cell fate

- Positive feedback to maintain cell fate

- Cell-cell interactions



Phenotype of wg mutant?

Being conservative –

- Developmental mechanisms can be reused

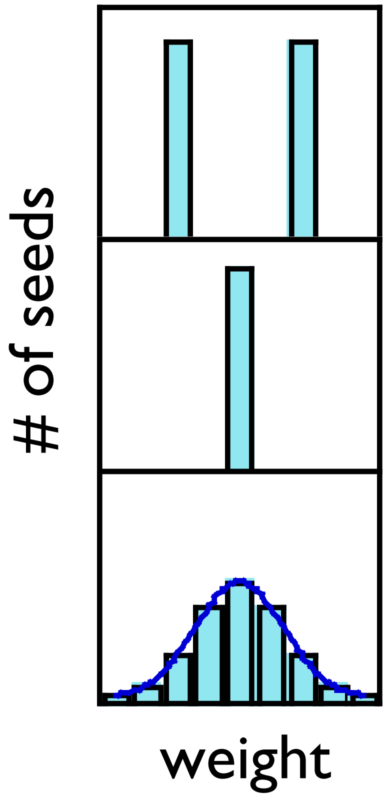
  e.g., hh and wg in fly leg

- Developmental mechanisms are often conserved across divergent species

Quantitative genetics

Many traits don't behave in a simple Mendelian fashion

e.g., seed weight



Parental:

F_1:

F_2:

Explanation:

Reminder: Snapdragon flower color inheritance (lecture 3)

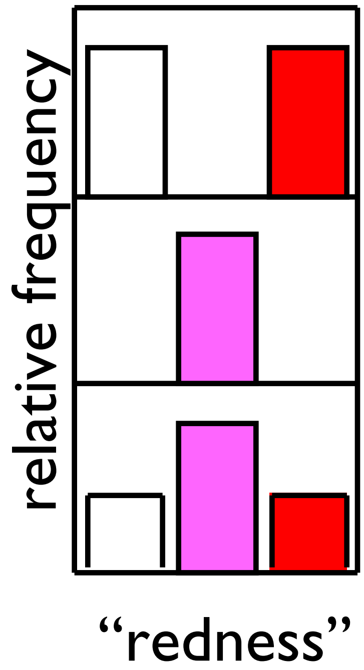
White x Red

↓

Pink

↓

1 red: 2 pink : 1 white



Basal level:

One increment of color:

Two increments:

Additive or **contributing** allele:

Non-additive or **non-contributing** allele:

Suppose there are **two genes** contributing to color?
Locus **A/a** and locus **B/b**

How many possible genotypes?

Non-additive alleles: **a, b**

☐ Basal level = no additive alleles =

☐ One additive allele:

☐ Two additive alleles:

☐ Three additive alleles:

☐ Four additive alleles:

Looking at a cross…

white x fully red

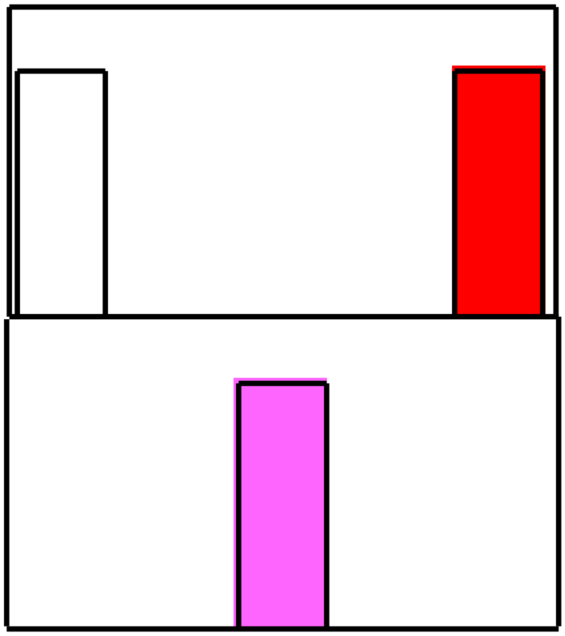
aabb x AABB

↓

AaBb Pink

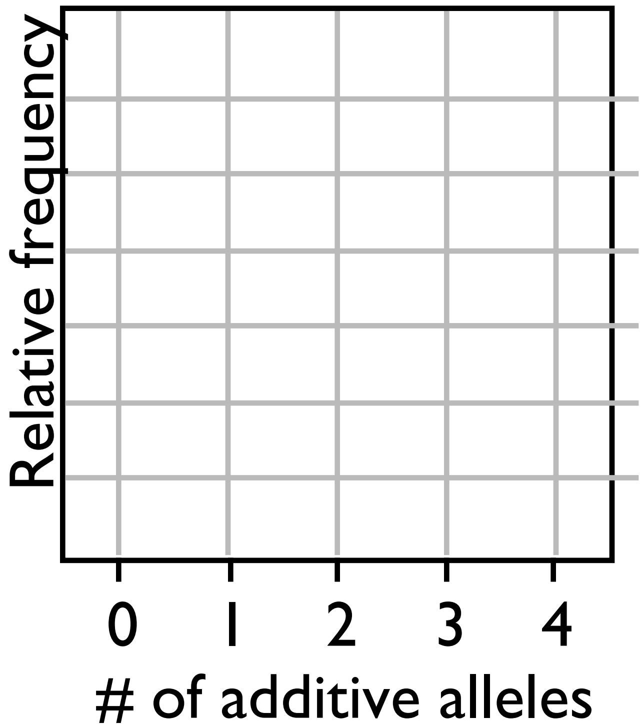
$F_1 \times F_1$

↓



Plot the number of additive alleles

	AB	Ab	aB	ab
AB				
Ab				
aB				
ab				



of genes = 2

of alleles = 4

of phenotypes =

distribution of additive allele frequencies:

fraction exhibiting extreme phenotype=

In general:

- # of genes:
- # of alleles
- # of phenotypes:
- distribution of additive allele frequencies:
- fraction exhibiting extreme phenotype:

Some assumptions:

Determining the number of polygenes (n):

1. Obtain true-breeders

2. Make F_1. Phenotype:

3. Cross F_1 to generate F_2. Phenotype:

4. Fraction of F_2 showing either extreme phenotype =

Why study quantitative genetics?

◇ Agriculture

◇ Human biology and health

◇ Studying evolution

Population genetics - I

Genetics 371B Lecture 33 | 1 Dec. 1999

a.k.a. Evolutionary Genetics

Why bother with this stuff?

The use of models

Some terminology

- **Genotype frequency**
  - P_{Aa}
  - P'_{Aa}
- **Allele frequency**
  - p_A
  - p'_A

The Random-Mating population

Assumptions

- ◇ Discrete generations
- ◇ Random mating
- ◇ Genotype frequencies in the two sexes are equal
- ◇ No mutation
- ◇ No immigration or emigration
- ◇ Genotypes are equally fertile
- ◇ No selection
- ◇ Infinite population size
- ◇ An autosomal locus

How do genotype frequencies change over time?

Starting genotype frequencies:

P_{AA}, P_{Aa}, P_{aa}

(Do we really want to do this?)

	AA	Aa	aa
AA			
Aa			
aa			

How do allele frequencies change over time?

Starting allele frequencies: p_A, p_a

- $p'_A =$
- $p'_a =$

What does this result tell us about the genotype frequencies?

- $P'_{AA} =$
- $P'_{Aa} =$
- $P'_{aa} =$

…These are the "Hardy-Weinberg frequencies"

How about the next generation?

Examining assumptions

- What if the two sexes **don't** have the same genotype frequencies?

Start with: p_{fA}, p_{mA}, p_{fa}, p_{ma}

$p'_{fA} =$ $\qquad$ $p'_{mA} =$

$p'_{fa} =$ $\qquad$ $p'_{ma} =$

Multiple alleles...

If the alleles are **a**, **b**, and **c**…

The possible genotypes are:

And their frequencies are:

And what about multiple loci?

- Unlinked loci

- Linked loci

Linkage disequilibrium

Population genetics - II

Genetics 371B Lecture 34 3 Dec. 1999

Evolution:

Quantifying genetic variation

Factors that alter allele frequencies

Genetic drift

Altered allele frequency due to random fluctuation…

Result: loss of variation (a.k.a. loss of heterozygosity)

Warwick Kerr, Sewall Wright

Drosophila experiment:

Wildtype x forked bristle mutant

+ = p = 0.5
forked (f) = q = 0.5

Pick at random:
4 males x 4 females, 100 parallel crosses

↓

Progeny

Expected: $p^2 + 2pq + q^2$

Observed, after 16 generations:

Consequence of random genetic drift:
heterozygotes are exchanged for homozygotes

…drift towards homozygosity

Ultimately:

How likely is the *Drosophila* result if 4000 males and females are chosen?

Calculating rate of loss due to drift

Rate of drift (loss of alleles) α

Loss of heterozygosity per generation =

Fraction heterozygous after *t* generations H_t …

Effect of inbreeding:

Founder effect: small population established from small initial sample

e.g., achromatopsia in Pingelap atoll

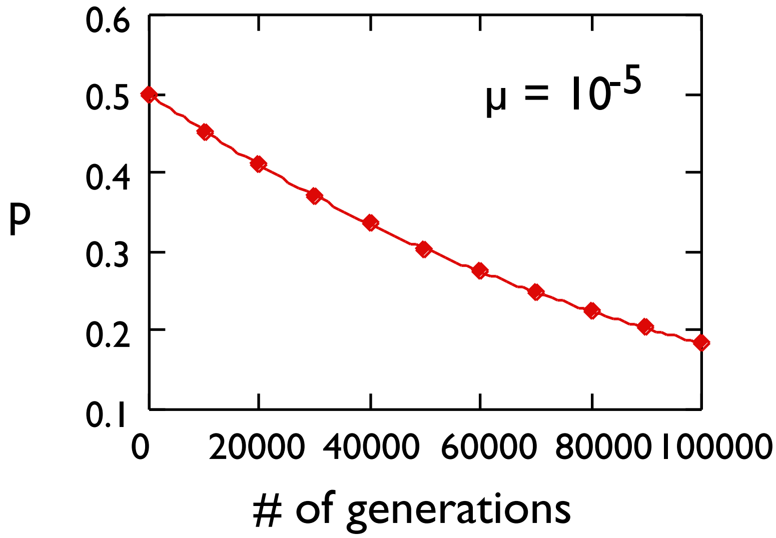
What counters the trend towards homozygosity?

Mutation

Mutation rate μ:

If initial frequency(A) = p0, then frequency(A) after 1 generation –

p_1 =



Mutation rate vs. genetic drift:

To counter loss of allele **a** (rate: 1/N) from drift…
would need mutation rate μ such that μ ≥ 1/N

Population genetics - III

Genetics 371B Lecture 35 6 Dec. 1999

Gene swamping – in absence of selection, most newly created alleles (rare!) will be lost from the population

Two possible outcomes (in closed population):
Get fixed, or get lost!

Chance of getting fixed: 1/2N
…why?

A molecular clock...

How many mutations get fixed per generation?

- Mutation rate per locus per generation = μ
- # of copies of the gene available to mutate = 2N
- # of mutations in the locus (in population) per generation =
- # of mutations that will be fixed in the population =

Migration

Movement of individuals between populations

How does it affect allele frequency?

If initial frequency of allele **A** in existing population= $\mathbf{p_0}$

and in immigrant population = $\mathbf{p_g}$

and **m** = coefficient of migration (fraction of population that is immigrant):

After 1 generation of immigration,

$$p_1 = (1-m)p_0 + mp_g$$

$$= p_0 + m(p_g - p_0)$$

Change in frequency of **A** = $p_1 - p_0 =$

How much migration is needed to counter genetic drift?

- Drift: 1/N
- Need: $\mathbf{m} \geq \mathbf{1/N}$
- or, need $\mathbf{mN} \geq \mathbf{1}$
- How many is that?

Selection

- **Fitness:** relative probability of survival and reproductive success due to a genetically inherited phenotype

- **What is selected,** the genotype or the phenotype?

- Selection may be –
  - directional

- stabilizing

- disruptive

The goals

How widespread is the problem?

How effective is treatment?

- Lifespan restored (completely corrective):
- Partial treatment:

Why is treatment so ineffective?

- mutant locus unknown
- irreversible pathology
- side effects

Best success:

…hence the drive to **find the genes**

Possible points of intervention

Mutant gene

↓

Mutant mRNA

↓

Mutant protein

↓

biochemical dysfunction

↓

Clinical phenotype

↓

Family/Society

Detection

- **Genetic counseling**
  - Medical diagnosis – the need for accuracy
  - Pedigree analysis ➡ Risk estimate
  - Counseling/followup

- **Prenatal or preimplantation testing**
  - Goals
  - Methods
    - *Amniocentesis*
    - *Chorionic villus sampling*
    - *Preimplantation testing*
  - Risks and ethical concerns

- **Genetic screening**
  - Purpose
  - Scope – who should be tested?
  - Testing –
    - *Deciding on a method*
    - *Pre-test and followup counseling*
  - Treatment options?

- Examples
  - *Screening for disease – PKU*

  - *Screening for carrier status – sickle cell disease*

- *Screening for carrier status – Tay-Sachs disease*

- *Is it always appropriate to screen? – the CF example*

Genetic diseases, cont'd

Treatment

- Surgical
- Drug treatment
    - *Sickle cell disease*
    - *Marfan syndrome*

- Dietary restriction
  - *PKU*
    - Unforeseen consequences
- Pharmacologic fiddling
  - *Hypercholesterolemia*
  - *Wilson disease*

- Replacing a missing gene product
  - *Diabetes*
  - *Growth hormone*
  - *ADA*
- Antisense therapy

- Gene therapy
  - *The theory*
  - *Methods*
  - *An example – ADA*

- *Concerns –*
  - Medical
  - Ethical
- Social tinkering (from lack of population concepts!)